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**From domestication to management: an
anthropological and ethological approach to the
welfare of domestic pigs under
naturalistic conditions**
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Abstract

By adopting an ethological and anthropological approach, this thesis investigates what 'domesticated' behavioural traits, in the social domain, can be relevant to assessing and improving animal welfare in extensive farming systems.

I expected that pigs, domesticated since the Neolithic, showed behavioural traits resulting from domestication process and selection against aggression. Initially, I focused on the detection of such traits to be used to assess pig welfare under naturalistic conditions. I examined the social play and aggression in piglets, as well as post-conflict strategies in adults. The structural differences analysis between play and real fighting indicated that both could help modulate competitive social dynamics depending on the context. Moreover, reconciliation (between individuals directly involved in conflict), triadic affiliation with third parties not involved in conflict (including consolation), and quadratic contacts between bystanders were observed in this research, and reduced anxiety-behaviour, assessed via a preliminary study not included in this thesis. Hence, post-conflict affiliation could play a key role in emotional regulation and social cohesion, with potential repercussions for animal welfare. These findings highlight that domestication does not simply result in reduced aggression but in greater behavioural flexibility, suggesting that it may allow for a more refined balancing of agonistic and affiliative behaviours in response to social dynamics. These aspects should be considered when assessing pig welfare.

Following, I applied the above behavioural phenomena to assess welfare in extensive farming systems. I analysed the effects of different management practices – such as the use of nose-clips to control rooting behaviour, group composition (all-female, all-male, mixed-sex), and environmental enrichment – on such behavioural phenomena. The results obtained by integrating behaviour and hormonal analyses, highlighted a transient increase in anxiety behaviours and stress (cortisol levels) following nose-clipping, with pre-application levels restored by the third week. The use of nose-clips to control invasive excavation behaviour proved to be an effective solution, reducing the rooting levels without increasing negative interactions (e.g. aggression) and decreasing positive social interactions (e.g. affiliation). Moreover, the mixed-sex group composition, while artificial and potentially less stable than natural groups (formed by related females or young males), showed higher levels of affiliation and post-conflict affiliation compared to other groups. This may reflect compensatory mechanisms to manage resource-related tensions. The introduction of environmental enrichment (e.g., straw) reduced anxiety behaviours and promoted non-invasive exploratory activities, although without significant effects on aggression levels. The interaction between social dynamics and environmental context underscores the importance of preserving the natural behavioural repertoire for effective emotional regulation.

In conclusion, this research has detected for the first time the structural properties of play compared to aggression, the presence and modulation of post-conflict

strategies, the effect of management measures on the social dimension of pigs. By doing so, this research contributes to a deeper understanding of social behaviours in pigs, suggesting that the ethological approach may serve as a useful tool for assessing and improving welfare in extensive farming systems, considering all the behavioural repertoire and cognitive potentials of the species. The anthropological approach allows the selection of behaviours that may result from domestication that related to increased tolerance and that may be more appropriate to understand pig social dynamics. The findings offer tools for enhancing management practices in naturalistic contexts, emphasising the complexity of emotional and social behavioural regulation.

Introduction

Domesticated mind and behaviour: an anthropological issue

The domestication of pigs (*Sus scrofa*) began approximately 10,000 years ago, independently in at least two distinct regions: northern Mesopotamia and China (Larson et al., 2007; Frantz et al., 2016; Price and Hongo, 2020). Generally, the traits most selected during domestication include lower levels of aggression and increased docility, which correspond to neotenic traits (Tennessen and Hudson, 1981; Zeder, 2012, 2015). These behavioural traits are often accompanied by physio-morphological characteristics (e.g., testosterone levels, cranial morphology), collectively referred to as the “domestication syndrome” (Trut, 1999; Wilkins et al., 2014; Zipser et al., 2014; Hansen Wheat et al., 2019; Lord et al., 2020). The changes also include cranial “pedomorphic” traits, such as a shortened snout, increased facial concavity, smaller cranial capacity, tooth crowding, and reduced tooth length, often linked to the overall size reduction observed in many domesticated species, including pigs (Clutton-Brock, 1988; Zeder, 2012; Owen et al., 2014; Lega et al., 2016). Experimental data suggest that these morphological changes may be triggered by selection along developmental pathways controlling the physiological systems responsible for reduced aggression (Trut, 1999; Zeder, 2012). Accordingly, the domestication process likely produced changes in the behavioural traits of domestic pigs, leading to a reduction in reactivity to threatening stimuli and increased social tolerance (Gustafsson et al., 1999; Trut, 1999; Künzl and Sachser, 1999; Lega et al., 2016; Hansen Wheat et al., 2019). Aggression levels appear to have played a role in the early stages of domestication, and genetic causes linked to variations in serotonin pathways are likely shared across domestic breeds (Leno-Colorado et al., 2017). Domestication may have fostered the emergence of behavioural traits that promote social cohesion while also enhancing competitiveness, reflecting on aggressive dynamics in social contexts (Gonyou, 2001; Jensen, 2002). In this regard, some behavioural and physiological elements could support the hypothesis of selection against aggression in domestic pigs. The reduction in aggressive response, the increase in positive social interactions, and the persistence of playful behaviours in young individuals suggest that processes similar to those hypothesised for other tolerant species may have influenced the behavioural evolution of pigs (Hare, 2017; Sánchez-Villagra and van Schaik, 2019). The opposition between play and aggression suggests that the maintenance of play in adulthood, though rare, might serve as a strategy to contain aggressive behaviours (Pellis and Iwaniuk, 2000; Cordoni and Norscia, 2024). Furthermore, as observed in other social species, selection for cooperation in environments with abundant resources could have favoured alternative strategies to aggression (de Waal, 2000a; Pisor and Surbeck, 2019). In other tolerant social species, the maintenance of juvenile traits, including playfulness and docility, has been associated with processes of self-domestication, in

which selection favoured strategies of cooperation and reduced aggressive reactivity (Hare, 2017; Wrangham, 2019). In general, compared to their wild counterparts, domestic animals often display increased levels of prosocial behaviours associated with positive emotions, such as sociability and playfulness, and reduced levels of reactive behaviours linked to negative emotions, such as stress, fear, and overt conflict (Trut, 1999; Künzl and Sachser, 1999; Himmeler et al., 2013; Hansen Wheat et al., 2019). Among domestic pigs, play sessions are extremely rare in adulthood (Newberry et al., 1988; Horback, 2014; Brown et al., 2018; Cordoni et al., 2021). However, play is the predominant form of social interaction among piglets (D'Eath and Turner, 2009; Camerlink et al., 2018; Špinka, 2017) and play fighting among piglets may substitute real fighting in certain circumstances to establish dominance relationships (Cordoni et al., 2021; Weller et al., 2020). A relevant aspect of pig domestication might involve a potential convergence in communication modules between pigs and humans, reflected in similar patterns of anxiety expression, post-conflict mechanisms and play. In this regard, similar anxiety-related behaviours have been observed between humans and non-human mammals (Blanchard et al. 2001), and the behavioural homologies between pigs and other social species, including humans, are evident in emotional regulation, particularly regarding anxiety (Norscia et al., 2021). Although domestication may have partially reduced reactivity to threatening stimuli, domestic pigs still show signs of stress when they are unable to escape from conflict or overcrowding (Goumon and Špinka, 2016). Domestic pigs express anxiety as a psychophysiological state, manifested through displacement activities that indirectly signal stress and emotional discomfort, such as vacuum chewing, rubbing, yawning and body shaking (Norscia et al., 2021). In extensive contexts, these indicators of anxiety increase following stressful events - such as intra-group aggression - and are modulated through affiliative mechanisms of social buffering: social contact between conspecifics rapidly reduces anxiety and restores stress levels to normal (Norscia et al., 2021). Therefore, anxious responses might be the result of evolutionary convergences between domestic pigs and other mammals with high cognitive potential, such as humans and non-human primates (Norscia et al. 2021), and could serve as useful indicators for assessing animal welfare in such contexts. Moreover, in social mammals, anxiety can be buffered through affiliative interactions between individuals, such as closeness, physical contact and allogrooming. This mechanism of social buffering has been observed in humans, non-human primates and non-primate mammals - including domestic pigs - with a reduction in displacement behaviours after grooming, play and post-conflict affiliative contacts (Davitz and Mason, 1955; Thorsteinsson et al., 1998; Hennessy et al., 2000; van Wolkenten et al., 2006; Aureli and Yates, 2010; Reimert et al., 2014; Burkett et al., 2016; Norscia et al., 2021). Social buffering is thus crucial for maintaining social cohesion, reducing tensions and promoting emotional welfare (Temple et al., 2011; Camerlink et al., 2014). These behaviours are particularly significant in managing pig welfare, especially in extensive farming systems where social behaviour is less influenced by managerial factors.

Furthermore, aspects related to play might contribute to understanding additional evolutionary convergences. Indeed, play fighting can be linked to the development of aggressive behaviour and dominance relationships in various mammalian species, including humans (Takahashi and Lore, 1983; Smith and Boulton, 1990; Paquette, 1994; Blumstein et al., 2013). In this sense, variations in both offensive and defensive behavioural patterns during play fighting can confer either a more competitive or a more cooperative nature to playful interactions (Bauer and Smuts, 2007; Cafazzo et al., 2018; Cordoni et al., 2021). Polyvagal theory proposes that the evolution of the mammalian autonomic nervous system provides the neurophysiological substrates for adaptive behavioural strategies, including the use of social play.

Despite these aspects, social animals must manage resource-related conflicts that inevitably arise within groups, and several species (including humans) have developed post-conflict behavioural strategies to preserve group integrity (de Waal, 2000a, b). Indeed post-conflict management strategies have been found in a wide array of social mammals (Aureli et al. 2002), including domestic animals (Schino, 1997). Therefore, an additional element potentially indicative of evolutionary convergence might be the ability to adopt effective post-conflict management strategies. In this sense, studying post-conflict strategies in domestic pigs could provide a valuable perspective for understanding conflict management dynamics in this species. By analysing how domestic pigs modulate social interactions after a conflict event, it is possible to highlight similarities and differences with other social mammals, thereby contributing to the understanding of the evolutionary basis of these mechanisms.

The domestic pig (*S. scrofa*) is a species of particular interest for studying animal behaviour and management in farming contexts. These animals exhibit complex socio-emotional interactions (Marino and Colvin, 2015) and, like wild boars, they live in stable groups composed of females with offspring or young males (Stolba and Wood-Gush, 1989; Gabor et al., 1999; Gonyou, 2001; D'Eath and Turner, 2008). Despite domestication, pigs retain a range of social behaviours similar to their wild counterparts, probably rooted in the species biology, showing a complex social organisation and a rich array of interactive behaviours (Stolba and Wood-Gush, 1989; Jensen, 2002; Norscia et al., 2021). In extensive farming contexts, domestic pigs, similarly to wild boars, engage in rooting and foraging activities, dedicating up to 52% of their time to these behaviours, particularly when new areas are available for exploration (Stolba and Wood-Gush, 1989; Studnitz et al., 2007). Behavioural differences between pigs and wild boars are largely attributable to environmental influences and interactions with humans, which have played a key role in the evolution of the species in response to domestication conditions (Stolba and Wood-Gush, 1989; Jensen, 2002). The practice of intensive farming raises significant environmental concerns due to substantial greenhouse gas emissions, groundwater pollution, and negative impacts on biodiversity (Leip et al. 2015; MacLeod et al. 2018). In the global context, the 2030 Agenda for Sustainable Development places particular emphasis on

agricultural practices that promote social, economic, and environmental sustainability, including specific measures to ensure animal welfare (United Nations, 2015). Furthermore, studies in the literature highlight that intensive farming systems aimed at maximising productivity per animal negatively affect the health and welfare of farm animals (Cobanovic and Magrin, 2023; Prunier et al. 2010). Therefore, such practices raise ethical and scientific questions regarding the respect of animals' psychological and behavioural needs, with direct impacts on their welfare. For instance, Broom et al. (2013) emphasise that a farming system can be considered sustainable if it is acceptable now and if its effects will remain acceptable in the future, particularly concerning resource availability, operational consequences, and the morality of actions.

Traditionally, research on the welfare of farm animals has primarily focused on welfare issues believed to be common in intensive systems. Conversely, the welfare of animals in extensive systems has received much less attention (Temple and Manteca, 2020). Indeed, extensive systems offer several welfare advantages over intensive systems—mainly in the behavioural domain—but it is also true that extensive systems can present welfare issues that are less common or less severe than those in intensive systems (Temple and Manteca, 2020). In particular, as highlighted by Villalba et al. (2016), animals are not always kept in environments where they evolved, and the unpredictability of environmental factors, coupled with human livestock management, does not always align with the animals' adaptive characteristics, potentially leading to welfare issues in extensive systems.

From an ethological perspective, behavioural research has helped better define the welfare conditions of farm animals and identify key behaviours that indicate states of welfare or stress (Dawkins, 2003; Orihuela, 2021). My master's thesis fits into this context, focusing on anxiety and anxiety-buffering mechanisms in domestic pigs. In my master's thesis and a subsequent published study (Norscia et al. 2021), the anxious behaviour of pigs was analysed in a semi-free environment at the "Ethical Farm Parva Domus" (Cavagnolo, Turin), with particular attention to post-aggressive anxiety modulation through affiliative behaviours. Observations revealed that, following intra-group aggression, anxiety manifested through displacement activities (vacuum chewing, scratching, head and body shaking, yawning; Norscia et al. 2021), but could be reduced through social interactions such as grooming and physical contact. These aspects suggest a complex social system that could be harnessed to improve welfare in extensive farming. Furthermore, this approach has underscored the importance of allowing animals to express their natural behavioural repertoire, reducing stress factors that underlie many problematic behaviours.

In line with modern perspectives, animal welfare is not limited to the absence of suffering but includes the ability to express natural behaviours and live in environments that meet their psychological and physiological needs, reducing negative experiences and promoting positive ones (Broom, 1991; Edgar et al., 2013). Anxiety, in particular, is often expressed behaviourally in association with a physiological stress response

(Troisi, 2002) and is especially relevant as it can interfere with an animal's ability to enjoy a good quality of life, thereby worsening welfare conditions (Blockhuis et al. 1998). In this sense, the ability to perform affiliative behaviours and interact positively with conspecifics is fundamental in the process of anxiety buffering, reducing its incidence and long-term consequences (Boissy et al., 2007). These aspects are crucial for defining effective management measures that improve the psychological welfare of animals.

Within this context, I developed the idea of identifying management strategies that, if properly implemented, could significantly reduce invasive excavation behaviours and improve animal welfare. The PON project "GREENPIG: Development of a new protocol to reduce the impact on biodiversity and increase animal welfare in domestic pigs raised in natural protected areas" aimed to further explore management practices designed to reduce invasive excavation behaviours through environmental improvements and social interactions in pigs. The goal was to develop measures that could be integrated into the daily management of farms, monitoring and assessing their effectiveness through an ethological and scientific approach.

Study Plan

My PhD project focuses on two fundamental aspects: the socio-emotional behavioural and communication features that may have been moulded by domestication and – based on that - the assessment of welfare in extensive farming systems. On the one hand, domestication has shaped the social behaviour of animals, influencing their expressive repertoire and group dynamics. On the other hand, extensive farming presents new challenges in managing animal welfare, requiring reliable indicators to properly evaluate the emotional and social state of pigs.

During my master's thesis, I investigated the expression and modulation of anxiety in pigs under semi-natural conditions, focusing on displacement activities and their variation in response to stressful events. I analysed how these behaviours were influenced by situations of social tension within the group and how post-event affiliation could contribute to reducing their incidence, suggesting a possible social buffering effect. My results were consistent with findings observed in different social species, including humans, and were published in a peer-reviewed journal (see Norscia et al., 2021 in the Appendix). This work served as the starting point for my doctoral research, in which I expanded the investigation to understand the behavioural mechanisms that emerge in response to selection against aggression, one of the central effects of domestication. Additionally, I conducted further research, not included in this thesis (Collarini et al. 2022, see chapter A2 in the Appendix), to evaluate how sows react to the isolation calls of their own piglets or those of other females. The results of this study highlight that increased levels of anxiety behaviour in sows were accompanied by an increased frequency of responses to piglet isolation calls. Furthermore, the findings

suggest that lactation and specific acoustic features play a key role in influencing these responses.

Understanding the behavioural repertoire of a species and enabling its expression can enhance welfare, as observed in farmed animals (Špinka, 2006; Kokocińska and Kaleta, 2016). An ethological approach allows for the evaluation of the physical, social, and behavioural conditions of animals, identifying behaviours indicative of stress or discomfort as well as those reflecting improved welfare, such as a reduction in aggressive behaviours and an increase in positive social interactions (Dellmeier, 1989; Koolhaas et al., 1999; Temple et al., 2011; Koolhaas and Van Reenen, 2016). Ethological observation of behaviours, including social interactions and signs of anxiety or relaxation, can therefore provide a precise means of assessing the effectiveness of management strategies, offering a concrete indication of animal welfare. In this regard, the domestic pig serves as a valuable model for studying behavioural changes induced by domestication. Although traits selected through domestication in the last thousands of years may have modulated some behavioural responses of the species, pigs have an evolutionary history of million of years and retain natural needs rooted in their biology (Gustafsson et al., 1999; Studnitz et al., 2003, 2007). If such needs are met, could promote optimal welfare. Understanding how pigs manifest anxiety and how to manage it provides a starting point. A deeper understanding of their social dynamics and post-conflict mechanisms could yield important insights for developing more sustainable and welfare-oriented management practices, particularly in extensive systems. In such contexts, where social behaviour is more natural and less influenced by management factors, studying pigs' emotional responses and social interactions is crucial for ensuring a welfare approach that is comprehensive.

Focusing on positive aspects of welfare, previous studies have suggested that certain behaviours can be potential indicators of good welfare or positive affective states in farmed animals (Boissy et al., 2007; Held and Špinka, 2011). Play, for example, is considered a rewarding activity (Trezza, Baarendse, and Vanderschuren, 2010; Vanderschuren, Achterberg, and Trezza, 2016) that plays a key role in fostering the development of physical, cognitive, and socio-emotional abilities and enhancing behavioural flexibility. Play involves and reorganises motor patterns typical of other behavioural systems (Pellis et al., 2019). Among the different types of play (including object and solitary play as well as various patterns of social play; Burghardt, 2005; Fagen, 1981), play fighting is arguably the most complex since animals interact physically and variably in close contact during play sessions (Palagi et al., 2016; Pellis and Pellis, 2017). Animals may combine different behavioural patterns during play fighting (Burghardt, 1998; Burghardt, 2005), including patterns typical of aggressive domains (Pellis and Pellis, 2017), making it challenging to clearly distinguish between play fighting and real fighting. Furthermore, in social mammals, play may play a role in conflict management, acting as a post-conflict reconciliation mechanism, as observed in bonobos and chimpanzees, where it helps re-establish social harmony after aggression (Muller and Mitani, 2005; Palagi et al., 2005).

Aims. The overarching aim of my PhD project was to develop an ethological approach to welfare assessment in pigs through two main lines of research:

1. Identifying behavioural measures linked to the selection against aggression operated during domestication: Analysing social play in piglets, a behaviour that may have been favoured over aggression; Investigating conflict management in adults, with particular attention to post-conflict mechanisms, such as reconciliation, triadic affiliation and quadratic contacts.
2. Assess welfare in extensive farming systems, by applying these measures (alongside behavioural, environmental, and hormonal indicators) to assess welfare in extensive farming systems, testing their impact under different conditions, such as the use of rooting control devices (*nose clip* vs. *no clip*), the potential effects of group composition (*all-male*, *all-female*, *mixed-sex* groups), and the impact of environmental enrichment (*absence* vs. *natural materials*).

Research Roadmap. To address these objectives, I followed a progressive approach, first investigating the relevant behavioural phenomena, possible result of domestication, and then applying them to welfare assessment:

- **Chapters 1 and 2 – The ontogeny of social play and aggression in piglets**
 - I compared social play in domestic piglets and wild boar hybrids to assess the possible impact of domestication (Chapter 1).
 - I analysed the structural differences between *play fighting* and *real fighting* to distinguish the two behaviours and evaluate their possible role in socio-emotional communications (Chapter 2).
- **Chapters 3 and 4 – Conflict management strategies in adults**
 - I examined the possible presence and dynamics of reconciliation and triadic affiliation in semi-wild pigs to understand what post-conflict social mechanisms are used by pigs to manage aggressive events (Chapter 3).
 - I investigated whether bystander affiliation had a role in reducing group tension after aggression (Chapter 4).
- **Chapters 5 and 6 – Application to extensive farming systems**
 - I tested the effects of nose clips on salivary cortisol levels and behavioral aspects such as anxiety-related behaviors, rooting and exploratory behaviors, and social interactions such as aggression, affiliation and post-conflict affiliation (Chapter 5).
 - I analysed the effects of group composition and environmental enrichment on behavioral aspects such as anxiety-related behaviors, rooting and exploratory behaviors, and social interactions such as aggression, affiliation and post-conflict affiliation (Chapter 6).

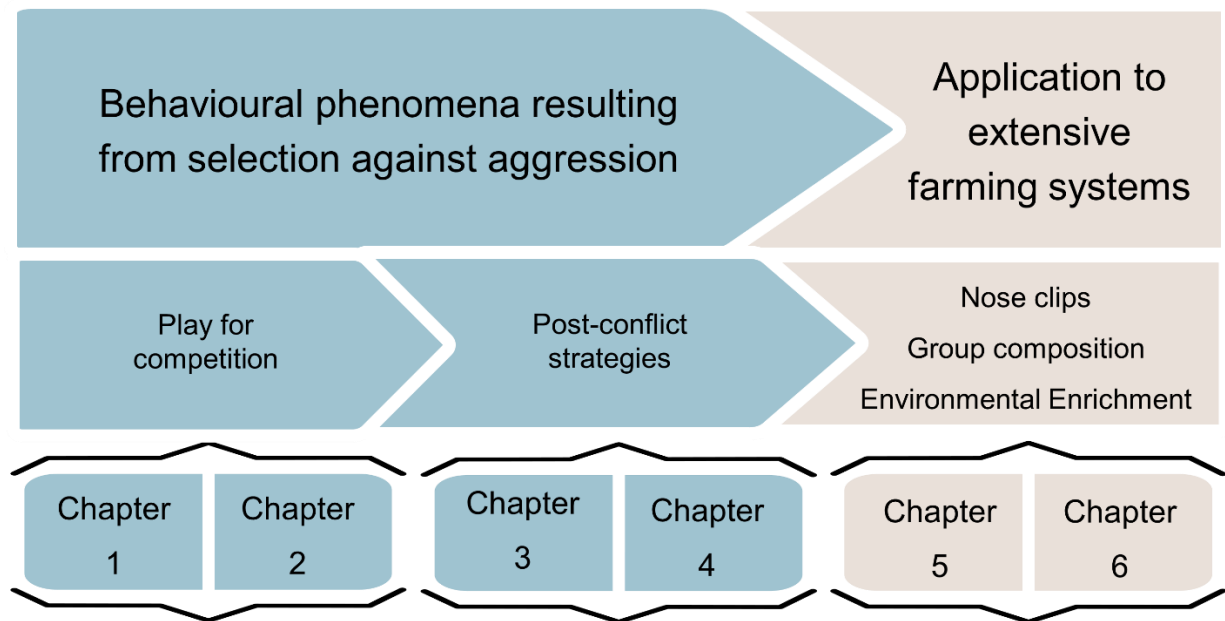


Figure 1. Graphical representation of the study plan

This research aims to explore how specific behavioural modules, such as competitive play and conflict management strategies, may serve as useful tools for assessing pig welfare. The ethological approach adopted could allow for an analysis of the impact of various environmental and management factors on social dynamics and the emotional state of the animals. This project seeks to provide a basis for the development of farming strategies that take both animal welfare and sector sustainability into account and the studies conducted within this PhD project resulted in multiple publications in peer-reviewed journals, reinforcing the validity of the proposed approach. Below, I briefly present the individual studies that are structured into the chapters of this thesis.

Chapter 1. Collarini, E., Gioia, M., Cordoni, G., & Norscia, I. (2022). Does the domestication syndrome apply to the domestic pig? Not completely. *Animals: An Open Access Journal from MDPI*, 12(18), 2458.

Domestication has led to a divergence in certain biological features that the domesticated form shows compared to the wild counterpart. This divergence involves profound changes not only in morphological and physiological traits but also, according to the domestication syndrome theory, in behavioural traits. Domestic animals are expected to display higher levels of affiliation and playfulness compared to their wild counterparts (Himmler et al., 2013; Hansen Wheat et al., 2019). However, this process may also have influenced the ontogeny of social play, reducing the frequency and complexity of play interactions in adults. In the first chapter, I explored the potential consequences of domestication syndrome by comparing the play behaviours of domestic pigs and hybrids of wild boar and domestic pigs. Hybrids represent an interesting model, as they may exhibit intermediate characteristics between domestic and wild forms. Specifically, I focused on the ontogeny of social play in piglets, aiming

to clarify whether domestication has affected the persistence of this behaviour and its social function.

I studied the behaviour of domestic piglets and wild boar hybrids by recording video sessions of aggressive and social play behaviours (primarily play fighting) of piglets (three litters) and hybrids (three litters) aged 6 to 50 days, raised in semi-natural conditions. I analysed the structure of the sessions using indices of asymmetry, variability, and distribution of patterns. I hypothesised that, due to domestication syndrome, domestic piglets would show more frequent, prolonged, and balanced play fighting sessions compared to wild boar-domestic hybrids. Additionally, I expected domestic pigs to exhibit lower levels of aggression, with shorter and less directional interactions than wild hybrids, reflecting the general tendencies of domestication syndrome. Understanding how domestication has shaped the ontogeny of social play provides key insights into how early-life experiences may contribute to the social competence of pigs. Since social play is linked to emotional regulation and social bonding, its study is crucial for assessing welfare. This sets the stage for the next chapter, where I further differentiate between playful and aggressive interactions in piglets, aiming to refine our understanding of their social dynamics.

Chapter 2. Cordoni, G., Collarini, E., Gioia, M., & Norscia, I. (2022). Play fighting versus real fighting in piglets (*Sus scrofa*): Similar patterns, different structure. *Behavioural Processes*, 203(104778), 104778.

Building on the findings from the previous chapter, where I examined the role of domestication in shaping social play, this chapter further explores the structural differences between play fighting and real fighting in piglets. Given the overlap in behavioural patterns used in both contexts, distinguishing between these two interaction types is essential for understanding the broader implications of social behaviour in pigs. The selection against aggression that characterizes the domestication process, can have led to the use of play as a form of non-aggressive way to compete. Play fighting in piglets includes one or more play markers absent in real fights, such as running, pivoting, head tossing, and shaking objects (Newberry and Wood-Gush, 1988; Rauw, 2013; Horback, 2014; Špinka, 2017). However, most of the behavioural patterns used by piglets during play fighting are also used during real fights: head-to-head wrestling, flank strikes, head blows to the playmate, pushing, lifting, and/or biting the partner (Rushen and Pajor, 1987; Newberry and Wood-Gush, 1988; Chaloupková et al., 2007; Šilerová et al., 2010; Horback, 2014; Pellis and Pellis, 2016; Brown et al., 2018; Cordoni et al., 2021). Thus, distinguishing between play fighting and real fights in piglets is not always straightforward. The considerable overlap of behavioural patterns between play and real fighting makes it difficult to distinguish them. However, these fine differences are crucial for assessing whether selection against aggression has promoted the use of play as an alternative strategy in domestic pigs.

In the second chapter, I used the same methods described in Chapter 1 and studied, for the first time, the structural differences between play fighting and real fights in piglets, aiming to distinguish social play from real fights given the similarities between these two types of interaction. I conducted video recordings of social interactions in piglets from three different litters. My hypothesis was that play-fighting sessions would be longer, more variable, and include more repeated movements compared to real fights. Another particularly interesting aspect of pigs' social behaviour is their post-conflict mechanisms. These mechanisms, widely documented in social mammal species, are essential for restoring the group's social homeostasis after a conflict and preventing violent escalation (De Waal, 2000b; Duboscq et al., 2014). From a welfare perspective, it is important to consider that positive social behaviour, including affiliative contacts, promotes cohesion and affiliation among conspecifics, while also mitigating the negative effects of stress by acting as an anxiety-buffering mechanism (Temple et al., 2011; Norscia et al., 2021). Thus, affiliative behaviours not only may help restore the emotional homeostasis of individuals but they may also be fundamental in maintaining stable relationships within the group, a crucial aspect for social welfare. Recognizing the structural differences between play fighting and real fights is essential for correctly interpreting social behaviour in pigs. This knowledge provides the basis for assessing how pigs regulate social tension. Furthermore, the persistence of play in piglets, despite its overlap with aggressive interactions, could reflect an evolutionary pathway shaped by selection against aggression. Play may serve as an alternative way to manage competition, potentially contributing to reduced reactive behaviours and increased social tolerance.

Chapter 3. Cordoni, G., Comin, M., Collarini, E., Robino, C., Chierito, E., & Norscia, I. (2023). Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: cognitive and functional implications. *Animal Cognition*, 26(2), 687–701.

Within social groups, resource conflicts inevitably occur and conflict management strategies include alternatives to aggression (de Waal, 2000). In this regard, a further aspect possibly reflecting the selection against aggression favoured by domestication (or self-domestication) could be conflict management. Social living, in humans and other animals, has led to the necessity of managing the conflicts that necessarily emerge within groups over resources, to avoid group disruption. To mitigate the negative impact of aggression, various post-conflict strategies can be implemented, including the "peaceful reunion" of former opponents (reconciliation; de Waal and van Roosmalen 1979; see the Appendix for definitions) or a "friendly contact" between one of the opponents and a third, uninvolved party (triadic affiliation; Romero et al. 2009, 2011; see the Appendix for definitions). Overall, post-conflict affiliation can reduce the anxiety generated by aggression in opponents and/or third parties involved in such affiliation (e.g. de Waal and Aureli, 1997; Fraser et al., 2008, 2009; Fraser and Bugnyar, 2010; see the Appendix for definitions). In this chapter, I analysed the post-

conflict behaviour of semi-wild pigs, investigating whether they display reconciliation and triadic affiliation behaviours (both solicited and unsolicited) following episodes of aggression. I observed a group of 104 pigs raised under semi-free-ranging conditions, collecting video data on conflict events and post-conflict behaviours, while accounting for known kinship relationships.

In this chapter, I hypothesised that pigs engage in reconciliation behaviours after conflicts, and that reconciliation would be more frequent between closely related individuals. Additionally, I anticipated that pigs would seek social support through triadic contacts, whether solicited or unsolicited, which could reduce anxiety and protect the victim from subsequent attacks. Specifically, I expected unsolicited contacts with the aggressor to lower the risk of further conflicts and for the victim to benefit from reduced anxiety.

Chapter 4. Norscia, I., Collarini, E., Robino, C., Chierto, E., & Cordoni, G. (2024). Witness for resolution: post-conflict quadratic affiliation in semi-free ranging pigs. *Current Zoology*, 70(2), 233–243.

Besides reconciliation and third-party affiliation (considered in Chapter 3), other post-conflict strategies may contribute to social stability within the group. While reconciliation and third-party affiliation directly involve former opponents or immediate social partners, conflict can have broader repercussions, affecting individuals beyond those directly involved in the aggressive event. Former opponents and third parties are not the only group members affected by aggression, as the impact of an aggressive event can reverberate throughout the entire group, increasing social tension (De Marco et al., 2010). In this regard, bystander interactions can be part of the conflict resolution process. When present, bystander affiliation can also help alleviate emotional arousal after witnessing a conflict (Judge and Mullen, 2005; see the Appendix for definitions). Therefore, as with other post-conflict strategies, bystander affiliation can contribute to restoring group homeostasis after a potentially disruptive event.

In this chapter, I investigated the presence of bystander affiliation in semi-wild pigs, exploring whether these animals exhibit such post-conflict behaviour. The study was conducted on the same group of 104 pigs observed in the previous chapter, collecting video data on conflict events and subsequent affiliative behaviours among individuals not directly involved in the conflict. I hypothesised that bystander affiliation, or interactions between individuals who were not involved in the conflict but interacted afterwards, would emerge in response to aggressive episodes, particularly when reconciliation or triadic affiliation behaviours were not already in place. I expected this type of affiliation to serve as a strategy to reduce anxiety and restore social balance within the group. Moreover, I predicted that bystander contacts would be more frequent among closely related individuals, as kinship can influence social behaviours in pigs.

By assessing whether these post-conflict mechanisms are present, to what extent, and with what dynamics (Chapters 3 and 4), it becomes possible to consider

them as valuable indicators of animal welfare. Understanding how pigs manage social tension and restore group balance provides crucial insights for welfare assessment, a perspective further explored in the following chapters.

For the first time in this thesis, specific behavioural phenomena are employed to assess welfare in an innovative way, integrating behavioural and physiological measures. This approach provides a more comprehensive understanding of pig welfare, moving beyond traditional assessments based on stress and aggression to include aspects of positive welfare.

Chapter 5. Collarini, E., Monte, O. D., Traversa, M., Mecarelli, E., Medana, C., Visentin, S., Cordonì, G., & Norscia, I. (2024). Nose-clip use in semi-free ranging pigs reduces rooting without disrupting affiliative behaviour or causing prolonged stress. *Animal: An International Journal of Animal Bioscience*, 101404.

Building on the findings of the previous chapters, welfare can be assessed by evaluating anxiety, aggression, and conflict management strategies. Additionally, other components contribute to the assessment, which will be introduced in this chapter. One key aspect is the role of exploratory and excavation behaviours in determining the overall welfare of free-ranging pigs and their impact on the environment. Among these, rooting behaviour is not only associated with foraging but also satisfies a need for exploration and interaction with the environment. Rooting is a vital part of the behavioural repertoire, representing a rewarding experience and meeting a behavioural need for pigs (Day et al., 1995; Studnitz et al., 2003, 2007). This behaviour, considered a source of animal welfare, is linked to reduced negative experiences such as frustration, boredom, and stress (Boissy et al., 2007; Kells, 2022). However, intensive rooting practices can lead to environmental problems, such as soil erosion and vegetation degradation (Edge et al., 2005). To mitigate such effects, farmers sometimes use nose rings to curb rooting behaviour, raising significant ethical concerns, particularly due to the frustration associated with restricting natural behaviour (Farm Animal Welfare Council, 1996; Studnitz et al., 2003). This trade-off reflects the complexity of animal welfare management, which must balance ecological and psychological needs. Therefore, it may be necessary to carefully evaluate alternatives to invasive practices.

In this chapter, I examined the use of nose clips as a less invasive alternative to rings for managing rooting behaviour in pigs. The pigs studied were raised under semi-free-ranging conditions, in a natural but enclosed environment. The study group consisted of 34 animals, divided into two homogeneous groups (17 subjects each): one experimental group with nose clips applied and one control group undergoing a similar procedure without clip application. Since pigs engage in social contacts through nose-to-nose interactions (Camerlink and Turner, 2013), nose clips could potentially affect both general affiliative and post-conflict affiliative behaviours, which, as previously described, play an anxiety-buffering role (Norscia et al., 2021). I collected data on anxiety-related behaviours (vacuum-chewing, scratching, yawning, body-

shaking); affiliative behaviours (physical contacts, nose-to-nose interactions) and post-conflict affiliative behaviours, aggression, rooting, and exploratory behaviours. Additionally, since cortisol levels increase in response to stressors (Beerda et al., 1998; Koopmans et al., 2005; Riek et al., 2019) and anxiety-related behaviours can be closely associated with the physiological response mediated by cortisol (Beerda et al., 2000; Thompson, 2011; Kubota et al., 2014; Rinaldi, 2019), I collected saliva samples to measure salivary cortisol levels. By combining behavioural indicators with a physiological measure of stress, this study offers a more integrated approach to welfare assessment. I expected cortisol levels to rise after clip application, followed by an increase in anxiety-related behaviours. Furthermore, considering the potential relationship between anxiety and aggression, I anticipated an increase in aggressive behaviours post-application. Additionally, I expected that the use of nose clips would reduce affiliative behaviours, particularly those involving nose-to-nose contact. Finally, I hypothesised a decrease in destructive rooting behaviours and an increase in less invasive exploratory behaviours.

Chapter 6. Collarini, E., Capponcelli, L., Pierdomenico, A., Cordoni, G., & Norscia, I. (2024). Balancing welfare and habitat damage in pigs (*Sus scrofa*) under extensive farming: an ethological approach for determining the effects of group composition and environmental enrichment. *Frontiers in Ethology*, 3.

In the previous chapter, I evaluated welfare in pigs by assessing the impact of the nose-clip application on behaviour and physiological stress responses. Here, I further investigate welfare by considering additional conditions, namely group composition and environmental enrichment. As described earlier, pig groups consist of associations of females or young males (Stolba and Wood-Gush, 1989; Gabor et al., 1999; Gonyou, 2001; D'Eath and Turner, 2008). Previous studies show that wild pigs can form groups characterised by strong social bonds, especially with related individuals, with very low levels of aggression (Podgórski et al., 2014). Additionally, in domestic pigs, establishing a stable social hierarchy can help avoid future conflicts and promote good social cohesion (Arey and Edwards, 1998; D'Eath and Turner, 2008). In this context, group composition might influence the behaviour of pigs raised in semi-free-ranging conditions. Therefore, in this final chapter, I studied the impact of group composition on pig behaviour, analysing single-sex and mixed groups. To test the effect of group composition, I collected data on groups with different compositions (all males, all females, and mixed), comparable in group size, age, and body size. Furthermore, I investigated the possible effect of environmental enrichment. A barren environment increases the risk of aggressive behaviours and stress (Petersen et al., 1995; Moinard et al., 2003; Brown et al., 2018), whereas the presence of environmental enrichments can promote exploratory behaviours and positive social interactions among individuals (Vanheukelom et al., 2012).

To assess the impact of environmental enrichments, I conducted additional data collection on the mixed group composition. During this period, three different types of

enrichments were provided in rotation: no enrichment, dry leaves, and straw. For both group composition and enrichment effects, I collected data on anxiety-related behaviours (vacuum-chewing, scratching, yawning, body-shaking); affiliative (physical contacts, nose-to-nose interactions) and post-conflict affiliative behaviours, aggression, rooting, and exploratory behaviours. By applying a multi-faceted approach that considers both group dynamics and enrichment, this study expands welfare assessment beyond individual physiological and behavioural measures, providing a broader understanding of how external factors shape social interactions and stress regulation. I expected that mixed groups (artificially created and potentially less stable) would display higher levels of negative social behaviours (such as anxiety and aggression) and rooting behaviours, as well as lower levels of affiliative and post-conflict affiliative behaviours compared to single-sex groups. Furthermore, I anticipated that the presence of environmental enrichments would lead to a decrease in negative events (such as anxiety and aggression) and rooting behaviours, alongside an increase in positive behaviours (affiliative and post-conflict affiliative) and exploratory behaviours.

Overall, this thesis takes an ethological approach to exploring how selection against aggression might be favoured by the domestication process and how this, in turn, might influence the social behaviour and welfare of domestic pigs. Through a step-by-step progression, the early chapters examine whether domestication shapes social play and aggressive interactions, considering the possibility that play acts as an alternative to direct competition. The focus then shifts to conflict management mechanisms, exploring whether and how reconciliation, post-conflict affiliation, and third-party social support contribute to restoring group homeostasis. Building on these foundations, the final chapters integrate behavioural and physiological assessments to evaluate the effects of management and environmental interventions on pig welfare in semi-natural conditions. By adopting this perspective, the research provides new insights into the social dynamics of pigs and assesses whether ethological indicators serve as innovative tools for evaluating and promoting animal welfare in sustainable farming systems.

References

- Arey, D. S., & Edwards, S. A. (1998). Factors influencing aggression between sows after mixing and the consequences for welfare and production. *Livestock Production Science*, 56(1), 61–70. [https://doi.org/10.1016/s0301-6226\(98\)00144-4](https://doi.org/10.1016/s0301-6226(98)00144-4)
- Aureli, F., Cords, M., & van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: A predictive framework. *Animal Behaviour*, 64(3), 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Aureli, F., & Yates, K. (2010). Distress prevention by grooming others in crested black macaques. *Biology Letters*, 6(1), 27–29. <https://doi.org/10.1098/rsbl.2009.0513>
- Bauer, E. B., & Smuts, B. B. (2007). Cooperation and competition during dyadic play in domestic dogs, *Canis familiaris*. *Animal Behaviour*, 73(3), 489–499. <https://doi.org/10.1016/j.anbehav.2006.09.006>
- Beerda, B., Schilder, M. B. H., van Hooff, J. A. R. A. M., de Vries, H. W., & Mol, J. A. (2000). Behavioural and hormonal indicators of enduring environmental stress in dogs. *Animal Welfare (South Mimms, England)*, 9(1), 49–62. <https://doi.org/10.1017/s0962728600022247>
- Beerda, B., Schilder, M. B. H., van Hooff, J. A. R. A. M., de Vries, H. W., & Mol, J. A. (1998). Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science*, 58(3–4), 365–381. [https://doi.org/10.1016/s0168-1591\(97\)00145-7](https://doi.org/10.1016/s0168-1591(97)00145-7)
- Blanchard, D. C., Hynd, A. L., Minke, K. A., Minemoto, T., & Blanchard, R. J. (2001). Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defense patterns of non-human mammals. *Neuroscience and Biobehavioral Reviews*, 25(7–8), 761–770. [https://doi.org/10.1016/s0149-7634\(01\)00056-2](https://doi.org/10.1016/s0149-7634(01)00056-2)
- Blumstein, D. T., Chung, L. K., & Smith, J. E. (2013). Early play may predict later dominance relationships in yellow-bellied marmots (*Marmota flaviventris*). *Proceedings. Biological Sciences*, 280(1759), 20130485. <https://doi.org/10.1098/rspb.2013.0485>
- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., ... & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, 92(3), 375–397. <https://doi.org/10.1016/j.physbeh.2007.02.003>
- Broom, D. M. (1991). Animal welfare: Concepts and measurement. *Journal of Animal Science*, 69(10), 4167–4175. <https://doi.org/10.2527/1991.69104167x>
- Broom, D. M., Galindo, F. A., & Murgueitio, E. (2013). Sustainable, efficient livestock production with high biodiversity and good welfare for animals. *Proceedings. Biological Sciences*, 280(1771), 20132025. <https://doi.org/10.1098/rspb.2013.2025>
- Brown, S. M., Bush, S. J., Summers, K. M., Hume, D. A., & Lawrence, A. B. (2018). Environmentally enriched pigs have transcriptional profiles consistent with neuroprotective

effects and reduced microglial activity. *Behavioural Brain Research*, 350, 6–15. <https://doi.org/10.1016/j.bbr.2018.05.015>

Brown, S. M., Peters, R., Nevison, I. M., & Lawrence, A. B. (2018). Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. *Applied Animal Behaviour Science*, 198, 36–43. <https://doi.org/10.1016/j.applanim.2017.09.018>

Burghardt, G. M. (1998). The evolutionary origins of play revisited: Lessons from turtles. In M. Bekoff & J. A. Byers (Eds.), *Animal Play* (pp. 1–26). Cambridge: Cambridge University Press.

Burghardt, G. M. (2005). *The genesis of animal play: Testing the limits*. Cambridge, MA: Bradford Books.

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B. M., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science (New York, N.Y.)*, 351(6271), 375–378. <https://doi.org/10.1126/science.aac4785>

Cafazzo, S., Marshall-Pescini, S., Essler, J. L., Virányi, Z., Kotrschal, K., & Range, F. (2018). In wolves, play behaviour reflects the partners' affiliative and dominance relationship. *Animal Behaviour*, 141, 137–150. <https://doi.org/10.1016/j.anbehav.2018.04.017>

Camerlink, I., & Turner, S. P. (2013). The pig's nose and its role in dominance relationships and harmful behaviour. *Applied Animal Behaviour Science*, 145(3–4), 84–91. <https://doi.org/10.1016/j.applanim.2013.02.008>

Camerlink, I., Farish, M., D'Eath, R. B., Arnott, G., & Turner, S. P. (2018). Long term benefits on social behaviour after early life socialization of piglets. *Animals: An Open Access Journal from MDPI*, 8(11), 192. <https://doi.org/10.3390/ani8110192>

Camerlink, I., Turner, S. P., Ursinus, W. W., Reimert, I., & Bolhuis, J. E. (2014). Aggression and affiliation during social conflict in pigs. *PloS One*, 9(11), e113502. <https://doi.org/10.1371/journal.pone.0113502>

Carter, C. S., Izja Lederhendler, I., & Kirkpatrick, B. (1999). *The integrative neurobiology of affiliation*. London, England: MIT Press.

Chaloupková, H., Illmann, G., Bartoš, L., & Špinka, M. (2007). The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Applied Animal Behaviour Science*, 103(1–2), 25–34. <https://doi.org/10.1016/j.applanim.2006.04.020>

Clutton-Brock, J. (1988). *Domesticated animals: their origin and evolution*. Unwin Hyman.

Čobanović, N., & Magrin, L. (2023). Editorial: Health and welfare problems of farm animals: Prevalence, risk factors, consequences and possible prevention solutions. *Frontiers in Veterinary Science*, 10. <https://doi.org/10.3389/fvets.2023.1238852>

Cordoni, G., Gioia, M., Demuru, E., & Norscia, I. (2021). The dark side of play: Play fighting as a substitute for real fighting in domestic pigs, *Sus scrofa*. *Animal Behaviour*, 175, 21–31. <https://doi.org/10.1016/j.anbehav.2021.02.016>

Cordoni, G., & Norscia, I. (2024). Nuancing “emotional” social play: Does play behaviour always underlie a positive emotional state? *Animals: An Open Access Journal from MDPI*, 14(19). <https://doi.org/10.3390/ani14192769>

Davitz, J. R., & Mason, D. J. (1955). Socially facilitated reduction of a fear response in rats. *Journal of Comparative and Physiological Psychology*, 48(3), 149–151. <https://doi.org/10.1037/h0046411>

Dawkins, M. S. (2003). Behaviour as a tool in the assessment of animal welfare. *Zoology (Jena, Germany)*, 106(4), 383–387. <https://doi.org/10.1078/0944-2006-00122>

Day, J., Spooler, H., & Edwards, S. (2001). Straw as environmental enrichment: Which properties do growing pigs find behaviourally rewarding. *CIGR Seminar on Animal Welfare Considerations in Livestock Housing Systems*. Retrieved from <https://eprints.ncl.ac.uk/54633>

de Waal, F.B.M. (2000a). Primates—a natural heritage of conflict resolution. *Science*, 289(5479), 586–590.

de Waal, F. B. M. (2000b). The first kiss: Foundations of conflict resolution research in animals. In F. Aureli (Ed.), *Natural conflict resolution* (Vol. 409, pp. 15–33). University of California Press.

de Waal, F. B. M., & Aureli, F. (1997). Conflict resolution and distress alleviation in monkeys and apes. In C. S. Carter (Ed.), *The integrative neurobiology of affiliation* (pp. 317–328). New York Academy of Sciences.

de Waal, F. B. M., & van Roosmalen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioral Ecology and Sociobiology*, 5(1), 55–66. <https://doi.org/10.1007/bf00302695>

Death, R. D., & Turner, S. P. (2007). The natural behaviour of the pig. In J. Marchant-Forde (Ed.), *The Welfare of Pigs* (pp. 13–45). Springer.

Dellmeier, G. R. (1989). Motivation in relation to the welfare of enclosed livestock. *Applied Animal Behaviour Science*, 22(2), 129–138. [https://doi.org/10.1016/0168-1591\(89\)90049-x](https://doi.org/10.1016/0168-1591(89)90049-x)

Duboscq, J., Agil, M., Engelhardt, A., & Thierry, B. (2014). The function of postconflict interactions: New prospects from the study of a tolerant species of primate. *Animal Behaviour*, 87, 107–120. <https://doi.org/10.1016/j.anbehav.2013.10.018>

Edge, H. L., Bulman, C. A., & Edwards, S. A. (2005). Alternatives to nose-ringing in outdoor sows: The provision of root crops. *Applied Animal Behaviour Science*, 92(1–2), 15–26. <https://doi.org/10.1016/j.applanim.2004.10.021>

Fagen, R. (1982). Skill and flexibility in animal play behavior. *The Behavioral and Brain Sciences*, 5(1), 162–162. <https://doi.org/10.1017/s0140525x00011006>

Farm Animal Welfare Council (FAWC) report on the welfare of pigs kept outdoors. (1996, June 1). Retrieved November 22, 2024, from <https://www.gov.uk/government/publications/fawc-report-on-the-welfare-of-pigs-kept-outdoors>

- Frantz, L., Meijaard, E., Gongora, J., Haile, J., Groenen, M. A. M., & Larson, G. (2016). The evolution of Suidae. *Annual Review of Animal Biosciences*, 4(1), 61–85. <https://doi.org/10.1146/annurev-animal-021815-111155>
- Fraser, O. N., & Bugnyar, T. (2010). Do ravens show consolation? Responses to distressed others. *PLoS One*, 5(5), e10605. <https://doi.org/10.1371/journal.pone.0010605>
- Fraser, O. N., Koski, S. E., Wittig, R. M., & Aureli, F. (2009). Why are bystanders friendly to recipients of aggression? *Communicative & Integrative Biology*, 2(3), 285–291. <https://doi.org/10.4161/cib.2.3.8718>
- Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*, 105(25), 8557–8562. <https://doi.org/10.1073/pnas.0804141105>
- Gabor, T. M., Hellgren, E. C., Van Den Bussche, R. A., & Silvy, N. J. (1999). Demography, sociospatial behaviour and genetics of feral pigs (*Sus scrofa*) in a semi-arid environment. *Journal of Zoology*, 247(3), 311–322. <https://doi.org/10.1017/s0952836999003039>
- Gonyou, H. W. (2001). The social behaviour of pigs. In *Social behaviour in farm animals* (pp. 147–176). CABI Publishing.
- Goumon, S., & Špinka, M. (2016). Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Animal Cognition*, 19(3), 501–511. <https://doi.org/10.1007/s10071-015-0950-5>
- Gustafsson, M., Jensen, P., de Jonge, F. H., & Schuurman, T. (1999). Domestication effects on foraging strategies in pigs (*Sus scrofa*). *Applied Animal Behaviour Science*, 62(4), 305–317. [https://doi.org/10.1016/s0168-1591\(98\)00236-6](https://doi.org/10.1016/s0168-1591(98)00236-6)
- Hansen Wheat, C., Fitzpatrick, J. L., Rogell, B., & Temrin, H. (2019). Behavioural correlations of the domestication syndrome are decoupled in modern dog breeds. *Nature Communications*, 10(1), 2422. <https://doi.org/10.1038/s41467-019-10426-3>
- Hare, B., Wobber, V., & Wrangham, R. (2012). The self-domestication hypothesis: Evolution of bonobo psychology is due to selection against aggression. *Animal Behaviour*, 83(3), 573–585. <https://doi.org/10.1016/j.anbehav.2011.12.007>
- Held, S. D. E., & Špinka, M. (2011). Animal play and animal welfare. *Animal Behaviour*, 81(5), 891–899. <https://doi.org/10.1016/j.anbehav.2011.01.007>
- Hennessy, M. B., Maken, D. S., & Graves, F. C. (2000). Consequences of the presence of the mother or unfamiliar adult female on cortisol, ACTH, testosterone and behavioral responses of periadolescent guinea pigs during exposure to novelty. *Psychoneuroendocrinology*, 25(6), 619–632. [https://doi.org/10.1016/s0306-4530\(00\)00014-7](https://doi.org/10.1016/s0306-4530(00)00014-7)
- Himmler, B. T., Stryjek, R., Modlinska, K., Derksen, S. M., Pisula, W., & Pellis, S. M. (2013). How domestication modulates play behavior: A comparative analysis between wild rats and a laboratory strain of *Rattus norvegicus*. *Journal of Comparative Psychology*, 127(4), 453–464. <https://doi.org/10.1037/a0032187>

Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2(2), 186. <https://doi.org/10.12966/abc.05.08.2014>

Jensen, P. (Ed.). (2002). *The ethology of domestic animals: An introductory text*. CABI Publishing.

Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, 69(6), 1345–1355. <https://doi.org/10.1016/j.anbehav.2004.08.016>

Kells, N. J. (2022). Review: The Five Domains model and promoting positive welfare in pigs. *Animal: An International Journal of Animal Bioscience*, 16(Suppl 2), 100378. <https://doi.org/10.1016/j.animal.2021.100378>

Kokocińska, A., & Kaleta, T. (2016). The role of ethology in animal welfare. *Animal Science and Genetics*, 12(1), 49–62. <https://doi.org/10.5604/01.3001.0013.6981>

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., ... Blokhuis, H. J. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, 23(7), 925–935. [https://doi.org/10.1016/s0149-7634\(99\)00026-3](https://doi.org/10.1016/s0149-7634(99)00026-3)

Koolhaas, J. M., & Van Reenen, C. G. (2016). ANIMAL BEHAVIOR AND WELL-BEING SYMPOSIUM: Interaction between coping style/personality, stress, and welfare: Relevance for domestic farm animals. *Journal of Animal Science*, 94(6), 2284–2296. <https://doi.org/10.2527/jas.2015-0125>

Koopmans, S. J., Ruis, M., Dekker, R., van Diepen, H., Korte, M., & Mroz, Z. (2005). Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. *Physiology & Behavior*, 85(4), 469–478. <https://doi.org/10.1016/j.physbeh.2005.05.010>

Kubota, N., Amemiya, S., Yanagita, S., Nishijima, T., & Kita, I. (2014). Emotional stress evoked by classical fear conditioning induces yawning behavior in rats. *Neuroscience Letters*, 566, 182–187. <https://doi.org/10.1016/j.neulet.2014.02.064>

Künzl, C., & Sachser, N. (1999). The behavioral endocrinology of domestication: A comparison between the domestic guinea pig (*Cavia aperea* f. *porcellus*) and its wild ancestor, the cavy (*Cavia aperea*). *Hormones and Behavior*, 35, 28–37.

Larson, G., Albarella, U., Dobney, K., Rowley-Conwy, P., Schibler, J., Tresset, A., ... Cooper, A. (2007). Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 104(39), 15276–15281. <https://doi.org/10.1073/pnas.0703411104>

Lega, C., Raia, P., Rook, L., & Fulgione, D. (2016). Size matters: A comparative analysis of pig domestication. *The Holocene*, 26(2), 327–332. <https://doi.org/10.1177/0959683615596842>

Leno-Colorado, J., Hudson, N. J., Reverter, A., & Pérez-Enciso, M. (2017). A pathway-centered analysis of pig domestication and breeding in Eurasia. *G3: Genes, Genomes, Genetics*, 7(7), 2171–2184. <https://doi.org/10.1534/g3.117.042671>

MacLeod, M. J., Vellinga, T., Opio, C., Falcucci, A., Tempio, G., Henderson, B., ... Gerber, P. J. (2018). Invited review: A position on the Global Livestock Environmental Assessment Model (GLEAM). *Animal: An International Journal of Animal Bioscience*, 12(2), 383–397. <https://doi.org/10.1017/s1751731117001847>

Marino, L., & Colvin, C. M. (2015). Thinking pigs: A comparative review of cognition, emotion, and personality in *Sus domesticus*. *International Journal of Comparative Psychology*, 28. <https://doi.org/10.46867/ijcp.2015.28.00.04>

Moinard, C., Mendl, M., Nicol, C. J., & Green, L. E. (2003). A case control study of on-farm risk factors for tail biting in pigs. *Applied Animal Behaviour Science*, 81(4), 333–355. [https://doi.org/10.1016/s0168-1591\(02\)00276-9](https://doi.org/10.1016/s0168-1591(02)00276-9)

Newberry, R. C., Wood-Gush, D. G., & Hall, J. W. (1988). Playful behaviour of piglets. *Behavioural Processes*, 17(3), 205–216. [https://doi.org/10.1016/0376-6357\(88\)90004-6](https://doi.org/10.1016/0376-6357(88)90004-6)

Newberry, R. C., & Wood-Gush, D. G. M. (1988). Development of some behaviour patterns in piglets under semi-natural conditions. *Animal Science*, 46(1), 103–109. <https://doi.org/10.1017/s0003356100003160>

Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>

Orihuela, A. (2021). Review: Management of livestock behavior to improve welfare and production. *Animal: An International Journal of Animal Bioscience*, 15(Suppl 1), 100290. <https://doi.org/10.1016/j.animal.2021.100290>

Owen, J., Dobney, K., Evin, A., Cucchi, T., Larson, G., & Strand Vidarsdottir, U. (2014). The zooarchaeological application of quantifying cranial shape differences in wild boar and domestic pigs (*Sus scrofa*) using 3D geometric morphometrics. *Journal of Archaeological Science*, 43, 159–167. <https://doi.org/10.1016/j.jas.2013.12.010>

Palagi, E., Burghardt, G. M., Smuts, B., Cordoni, G., Dall'Olio, S., Fouts, H. N., ... Pellis, S. M. (2016). Rough-and-tumble play as a window on animal communication: Social play and communication in animals. *Biological Reviews of the Cambridge Philosophical Society*, 91(2), 311–327. <https://doi.org/10.1111/brv.12172>

Paquette, D. (1994). Fighting and playfighting in captive adolescent chimpanzees. *Aggressive Behavior*, 20(1), 49–65. [https://doi.org/10.1002/1098-2337\(1994\)20:1<49::aid-ab2480200107>3.0.co;2-c](https://doi.org/10.1002/1098-2337(1994)20:1<49::aid-ab2480200107>3.0.co;2-c)

Pellis, S. M., & Iwaniuk, A. N. (2000). Adult–adult play in primates: Comparative analyses of its origin, distribution and evolution. *Ethology*, 106(12), 1083–1104. <https://doi.org/10.1046/j.1439-0310.2000.00627.x>

- Pellis, S. M., & Pellis, V. C. (2016). Play fighting in Visayan warty pigs (*Sus cebifrons*): Insights on restraint and reciprocity in the maintenance of play. *Behaviour*, 153(6–7), 727–747. <https://doi.org/10.1163/1568539x-00003346>
- Pellis, S. M., & Pellis, V. C. (2017). What is play fighting and what is it good for? *Learning & Behavior*, 45(4), 355–366. <https://doi.org/10.3758/s13420-017-0264-3>
- Pellis, S. M., Pellis, V. C., Pelletier, A., & Leca, J.-B. (2019). Is play a behavior system, and, if so, what kind? *Behavioural Processes*, 160, 1–9. <https://doi.org/10.1016/j.beproc.2018.12.011>
- Petersen, V., Simonsen, H. B., & Lawson, L. G. (1995). The effect of environmental stimulation on the development of behaviour in pigs. *Applied Animal Behaviour Science*, 45(3–4), 215–224. [https://doi.org/10.1016/0168-1591\(95\)00631-2](https://doi.org/10.1016/0168-1591(95)00631-2)
- Pisor, A. C., & Surbeck, M. (2019). The evolution of intergroup tolerance in nonhuman primates and humans. *Evolutionary Anthropology*, 28(4), 210–223. <https://doi.org/10.1002/evan.21793>
- Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PloS One*, 9(6), e99875. <https://doi.org/10.1371/journal.pone.0099875>
- Price, M., & Hongo, H. (2020). The archaeology of pig domestication in Eurasia. *Journal of Archaeological Research*, 28(4), 557–615. <https://doi.org/10.1007/s10814-019-09142-9>
- Prunier, A., Heinonen, M., & Quesnel, H. (2010). High physiological demands in intensively raised pigs: Impact on health and welfare. *Animal: An International Journal of Animal Bioscience*, 4(6), 886–898. <https://doi.org/10.1017/S175173111000008X>
- Rauw, W. M. (2013). A note on the consistency of a behavioral play marker in piglets. *Journal of Animal Science and Biotechnology*, 4(1), 33. <https://doi.org/10.1186/2049-1891-4-33>
- Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology & Behavior*, 129, 221–229. <https://doi.org/10.1016/j.physbeh.2014.02.059>
- Riek, A., Schrader, L., Zerbe, F., & Petow, S. (2019). Comparison of cortisol concentrations in plasma and saliva in dairy cattle following ACTH stimulation. *The Journal of Dairy Research*, 86(4), 406–409. <https://doi.org/10.1017/S0022029919000669>
- Rinaldi, G. (2019). The itch-scratch cycle: A review of the mechanisms. *Dermatology Practical & Conceptual*, 9(2), 90–97. <https://doi.org/10.5826/dpc.0902a03>
- Romero, T., Castellanos, M. A., & de Waal, F. B. M. (2011). Post-conflict affiliation by chimpanzees with aggressors: Other-oriented versus selfish political strategy. *PloS One*, 6(7), e22173. <https://doi.org/10.1371/journal.pone.0022173>
- Romero, T., Colmenares, F., & Aureli, F. (2009). Testing the function of reconciliation and third-party affiliation for aggressors in hamadryas baboons (*Papio hamadryas hamadryas*). *American Journal of Primatology*, 71(1), 60–69. <https://doi.org/10.1002/ajp.20619>

Rushen, J., & Pajor, E. (1987). Offence and defence in fights between young pigs (*Sus scrofa*). *Aggressive Behavior*, 13(6), 329–346. [https://doi.org/10.1002/1098-2337\(1987\)13:6<329::aid-ab2480130602>3.0.co;2-3](https://doi.org/10.1002/1098-2337(1987)13:6<329::aid-ab2480130602>3.0.co;2-3)

Sánchez-Villagra, M. R., & van Schaik, C. P. (2019). Evaluating the self-domestication hypothesis of human evolution. *Evolutionary Anthropology*, 28(3), 133–143. <https://doi.org/10.1002/evan.21777>

Schino, G. (1998). Reconciliation in domestic goats. *Behaviour*, 135(3), 343–356. <https://doi.org/10.1163/156853998793066302>

Šilerová, J., Špinka, M., Šárová, R., & Algers, B. (2010). Playing and fighting by piglets around weaning on farms, employing individual or group housing of lactating sows. *Applied Animal Behaviour Science*, 124(3–4), 83–89. <https://doi.org/10.1016/j.applanim.2010.02.003>

Smith, P. K., & Boulton, M. (1990). Rough-and-tumble play, aggression and dominance: Perception and behaviour in children's encounters. *Human Development*, 33(4–5), 271–282. <https://doi.org/10.1159/000276524>

Špinka, M. (2006). How important is natural behaviour in animal farming systems? *Applied Animal Behaviour Science*, 100(1–2), 117–128. <https://doi.org/10.1016/j.applanim.2006.04.006>

Špinka, M. (2017). Behaviour of pigs. In *The ethology of domestic animals: An introductory text* (pp. 214–227). CABI.

Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science (Penicuik, Scotland)*, 48(2), 419–425. <https://doi.org/10.1017/s0003356100040411>

Studnitz, M., Jensen, K. H., & Jørgensen, E. (2003). The effect of nose rings on the exploratory behaviour of outdoor gilts exposed to different tests. *Applied Animal Behaviour Science*, 84(1), 41–57. [https://doi.org/10.1016/s0168-1591\(03\)00144-8](https://doi.org/10.1016/s0168-1591(03)00144-8)

Studnitz, M., Jensen, M. B., & Pedersen, L. J. (2007). Why do pigs root and in what will they root? *Applied Animal Behaviour Science*, 107(3–4), 183–197. <https://doi.org/10.1016/j.applanim.2006.11.013>

Takahashi, L. K., & Lore, R. K. (1983). Play fighting and the development of agonistic behavior in male and female rats. *Aggressive Behavior*, 9(3), 217–227. [https://doi.org/10.1002/1098-2337\(1983\)9:3<217::aid-ab2480090303>3.0.co;2-4](https://doi.org/10.1002/1098-2337(1983)9:3<217::aid-ab2480090303>3.0.co;2-4)

Temple, D., & Manteca, X. (2020). Animal welfare in extensive production systems is still an area of concern. *Frontiers in Sustainable Food Systems*, 4. <https://doi.org/10.3389/fsufs.2020.545902>

Temple, D., Manteca, X., Velarde, A., & Dalmau, A. (2011). Assessment of animal welfare through behavioural parameters in Iberian pigs in intensive and extensive conditions. *Applied Animal Behaviour Science*, 131(1–2), 29–39. <https://doi.org/10.1016/j.applanim.2011.01.013>

Tennessen, T., & Hudson, R. J. (1981). Traits relevant to the domestication of herbivores. *Applied Animal Ethology*, 7(1), 87–102. [https://doi.org/10.1016/0304-3762\(81\)90054-7](https://doi.org/10.1016/0304-3762(81)90054-7)

Thompson, S. B. N. (2011). Born to yawn? Cortisol linked to yawning: A new hypothesis. *Medical Hypotheses*, 77(5), 861–862. <https://doi.org/10.1016/j.mehy.2011.07.056>

Thorsteinsson, E. B., James, J. E., & Gregg, M. E. (1998). Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology: Official Journal of the Division of Health Psychology, American Psychological Association*, 17(5), 436–444. <https://doi.org/10.1037/0278-6133.17.5.436>

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: Pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences*, 31(10), 463–469. <https://doi.org/10.1016/j.tips.2010.06.008>

Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress (Amsterdam, Netherlands)*, 5(1), 47–54. <https://doi.org/10.1080/102538902900012378>

Trut, L. N. (1999). Early canid domestication: The farm-fox experiment: Foxes bred for tamability in a 40-year experiment exhibit remarkable transformations that suggest an interplay between behavioral genetics and development. *American Scientist*, 87, 160–169.

United Nations. (2015). *Transforming our world: The 2030 Agenda for Sustainable Development*. Retrieved from <https://sdgs.un.org>

van Wolkenten, M. L., Davis, J. M., Gong, M. L., & de Waal, F. B. M. (2006). Coping with acute crowding by *Cebus apella*. *International Journal of Primatology*, 27(5), 1241–1256. <https://doi.org/10.1007/s10764-006-9070-z>

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience and Biobehavioral Reviews*, 70, 86–105. <https://doi.org/10.1016/j.neubiorev.2016.07.025>

Vanheukelom, V., Driessen, B., & Geers, R. (2012). The effects of environmental enrichment on the behaviour of suckling piglets and lactating sows: A review. *Livestock Science*, 143(2–3), 116–131. <https://doi.org/10.1016/j.livsci.2011.10.002>

Villalba, J. J., Manteca, X., Vercoe, P. E., Maloney, S. K., & Blache, D. (2016). Integrating nutrition and animal welfare in extensive systems. In *Animal Welfare* (pp. 135–163). Springer International Publishing.

Wilkins, A. S., Wrangham, R. W., & Fitch, W. T. (2014). The “domestication syndrome” in mammals: A unified explanation based on neural crest cell behavior and genetics. *Genetics*, 197(3), 795–808. <https://doi.org/10.1534/genetics.114.165423>

Wrangham, R. W. (2019). Hypotheses for the evolution of reduced reactive aggression in the context of human self-domestication. *Frontiers in Psychology*, 10, 1914. <https://doi.org/10.3389/fpsyg.2019.01914>

Zeder, M. A. (2012). The domestication of animals. *Journal of Anthropological Research*, 68(2), 161–190. <https://doi.org/10.3998/jar.0521004.0068.201>

Zeder, M. A. (2015). Core questions in domestication research. *Proceedings of the National Academy of Sciences of the United States of America*, 112(11), 3191–3198. <https://doi.org/10.1073/pnas.1501711112>

1. Does the Domestication Syndrome Apply to the Domestic Pig? Not Completely

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Abstract

The ‘domestication syndrome’ defines a suite of features that domesticated animals possess as the result of the artificial selection operated by *Homo sapiens* since the Neolithic. An interesting anthropological question is whether such features, including increased tameness and reduced aggression, apply to all domesticated forms. We investigated this issue in the domestic pig (*Sus scrofa*). We video-recorded and analysed aggression and social play (mostly play-fighting) sessions from piglets (three litters; $n = 24$) and wild boar hybrids (domestic pig mother x wild boar father; three litters; $n = 27$) from 6–50 days of age, raised in the same woodland/grassland habitat and extensive farming management (ethical farm ‘Parva Domus’, Cavagnolo, Torino). Play and aggression session structure was assessed via Asymmetry (AI; offensive/defensive pattern balance), Shannon (H' ; pattern variability), and Pielou (J; pattern evenness) indices. We found that piglets played more (especially after the 20th day of life) and engaged in less variable and uniform sessions than wild boar hybrids. Compared to hybrids, piglets showed less variable but more frequent (especially when approaching weaning) and asymmetrical aggressive events. Thus, the domestication syndrome does not seem to fully apply to either social play or aggression, possibly because artificial selection has produced greater tameness of pigs towards humans than towards conspecifics.

1.1 Introduction

Over the past 11,000 years humans have domesticated a wide range of animal species as livestock and household pets (Mignon-Grasteau et al., 2005). Domestication is an evolutionary process through which animals are artificially selected and become adapted to humans and to the captive environment (Price, 1984; Trut et al., 2009). Via this process, humans modulate different aspects of the natural history of the domesticated species, including distribution, breeding, diet, and behaviour (Clutton-Brock, 1992; Zeder, 2012, 2015). From a behavioural point of view, the most selected characteristics undergoing domestication are low levels of aggression and increased tameness, which correspond to neotenuous features (Zeder, 2012; Del Savio and Mameli, 2020; Murphy, 1985; Tennessen and Hudson, 1981; Theofanopoulou et al., 2017; Trut, 1999). For example, dogs are neotenuous compared to their wild counterparts-the wolves (Goodwin et al., 1997; Mcnamara, 1997) - because artificial selection has favoured individuals showing more playful and less aggressive behaviours (Coppinger et al., 2010; Morey, 1994; Waller et al., 2013). Such a suite of behavioural traits - often accompanied by physio-morphological features (e.g., testosterone levels, cranial morphology) - is known as 'domestication syndrome' (Lord et al., 2020; Zipser et al., 2014). As a result, when compared with their wild counterparts, domesticated animals commonly express increased levels of prosocial behaviours associated with positive emotions, such as sociability and playfulness, and lowered levels of reactive behaviours associated with negative emotions, such as stress, fear, and overt conflict (Hansen Wheat et al., 2019; Hare et al., 2012; Himmler et al., 2013; Künzl and Sachser, 1999; Trut, 1998).

As concerns household pets, increases in tameness seem to have characterised the domestication of the cat (*Felis catus*), starting from wild species with more docile temperaments (probably *Felis libyca*) (Serpell, 1989). The behavioural modification from fear-aggressiveness to tameness is also a trait that has probably marked the initial domestication of dogs (*Canis lupus familiaris*), leading to increased playfulness and decreased persistent aggression in modern breeds (although not in a consistent way across time and breeds; Hansen Wheat et al., 2019; Zhang et al., 2020). Moreover, compared to wild caviae (*Cavia aperea*), domestic guinea pigs (*Cavia porcellus*) are characterised by higher levels of social activity and lower levels of exploration, risk-taking, and cortisol reactivity (Zipser et al., 2014). As concerns livestock, the increased docility and suppression of the fight-or-flight response seem to be traits that have been selected during the domestication of goats (*Capra hircus*) and sheep (*Ovis aries*) (compared to wild ancestors, such as the Persian wild goat *Capra aegagrus* or West Asiatic mouflon *Ovis orientalis*, respectively; Clutton-Brock, 1992; Zohary et al., 1998). Despite these examples, the literature reports very few case studies that directly compare the behaviour of domestic species with the behaviour of their wild counterparts in order to quantitatively assess - from an anthropological perspective - the behavioural differences possibly associated with the domestication syndrome. To fill in part this gap, we carried out a direct behavioural comparison between wild boar hybrids and domestic pigs (*Sus scrofa*). The domestic pig is an

excellent model to investigate because its initial domestication (which occurred independently in at least two regions, Northern Mesopotamia and China) dates to around 10,000 years ago (Price and Hongo, 2020; Frantz et al., 2016; Larson et al., 2007). Domestic pigs show sociality and gregariousness, low reactions to humans, and low fear reactions (Mignon-Grasteau et al., 2005; Price, 1999; D'eath and Turner, 2009). Recently, Price and Hongo (2020) encouraged the adoption of a multimethod approach to shed light on the pig domestication process, which has led to genotypical/phenotypical adaptations to human management. We posit that the comparison between domestic and wild forms could be part of such a multimethod approach. As a matter of fact, although the behavioural repertoire of pigs is as rich as the repertoire of their wild counterpart (the wild boar; D'eath and Turner, 2009; Mendl, 1994; Stolba and Wood-Gush, 1989), it is possible to find differences caused by changes in sensitivity to the stimuli and there may be quantitative variations in the level of expression of certain behavioural patterns (D'eath and Turner, 2009; Stolba and Wood-Gush, 1989; Dellmeier and Friend, 1991; Jensen, 1995; Petersen et al., 1990). For example, wild boar and feral pigs (pigs that have lived in wild conditions for generations) are more reactive than domestic pigs to the presence of predators, including humans (Kurz and Marchinton, 1972). In this regard, the process of domestication probably produced changes in behavioural traits of domestic pigs (D'eath and Turner, 2009; Gustafsson et al., 1999a, 1999b, 1999; Špinka et al., 2000; Cordoni et al., 2021). Although one important selection criterion by early breeders was the fast growth rate (Lega et al., 2016), aggression levels seem to have played a role during the early stages of domestication and the genetic causes (linked to variations in the serotonin pathways) are likely shared across domestic breeds (Leno-Coloardo et al., 2017). Moreover, social traits that emerged in ancestral suids millions of years ago, have been altered by human selection in the Holocene during the domestication process, to favour docility (Price and Hongo, 2020).

In this study, we compared the aggressive and social play behaviour in three litters of piglets and three litters of wild boar x domestic pig hybrids (hereafter wild boar hybrids) raised under the same natural (woodland/grassland) and management conditions. The immature phase is particularly suitable because, although a certain degree of sociality is maintained in adults, especially in females, sociality tends to decrease with aging. As a matter of fact, social contacts including play, gradually decrease after the first phases of life (Gustafsson, 1999b; Horback, 2014; Petersen et al., 1989). The immature phase also reduces possible biases related to aspects of social life that may be learned from experience. To investigate what behavioural traits of pigs may have been moulded by the domestication process (which foresees increased docility and decreased aggression), we formulated the following predictions.

2.1.1. Prediction 1

During the early days of life, play behaviour, including play-fighting (or rough-and-tumble play), is the main social contact that occurs between piglets (Petersen et al. 1990; Cordoni et al. 2021; Newberry et al., 1988; Newberry and Wood-Gush, 1985).

Play-fighting helps piglets to develop the motor and social skills that are necessary to face opponents, enhance conflict resolution, individual recognition, group cohesion, and social bonds (Horback, 2014; Clouard et al., 2022, Newberry et al., 1988). Following the domestication syndrome, domesticated animals are expected to show higher levels of affiliation and playfulness compared to their wild counterparts (Hansen Wheat et al., 2019; Himmler et al., 2013). Hence, we posited that play-fighting would be more expressed in piglets than in wild boar hybrid pups (*Prediction 1a*). Successful playful interactions are characterized by long duration and high balance because subjects are more skilled in managing the high pattern variability typical of play (Burghardt, 2005, 2011; Aldis, 2013; Pellis and Pellis, 1998; Bekoff, 2001). Thus, if the domestication syndrome applies to the domestic pigs and domestic pigs are more 'skilled' players than their wild counterparts, we expected that piglets would be more capable to balance and prolong highly variable play fight sessions (*Prediction 1b*). Social play is a widespread behaviour that is mostly typical of immature subjects and decreases as age increases, also in pigs (Cordoni et al. 2021; Newberry and Wood-Gush, 1988). Hence, if the domestication syndrome applies to the domestic pigs and has led to the presence of behavioural neotenous traits, we expected that piglets - compared to wild boar hybrids - would show higher levels of social play at later stages of the lactation period, when weaning approaches (*Prediction 1c*).

2.1.2 Prediction 2

In adult pigs, conflicts can be observed to access food and establish hierarchy (D'eath and Turner, 2009). In piglets, aggressive behaviours can be observed since the very first days of life when they compete for the most productive udders (Cordoni et al. 2021; Newberry and Wood-Gush, 1985,1988). However, it is also possible to observe aggressive behaviour among immature subjects out of the context of lactation (Petersen et al., 1989). According to the domestication syndrome, domesticated animals should show lower levels of aggression compared to their wild counterparts (Gepts and Papa, 2002; Wilnkins and Wrangham, 2014; Wright, 2015). Hence, we expected to find lower levels of aggression in piglets compared to wild boar hybrids (*Prediction 2a*). In species with low tolerance levels, individuals fiercely compete to gain advantage over their groupmates, thus establishing higher ranking positions and obtaining priority access to desirable resources (De Boer et al., 2016, Norscia and Palagi, 2016; Koolhaas et al., 2013). In this respect, aggressive social groups should show real fights that are highly directional (unbalanced in favour of dominants) and predictable in terms of offensive/defensive patterns. Hence, if the domestic form is more 'peaceful' than the wild form, as predicted by the domestication syndrome, we expected that aggression in piglets would be shorter and less directional and predictable than in wild boar hybrids (*Prediction 2b*). Aggressive events increase as age increases and sexual maturity is approached, because aggression is used to access food and to set up dominance relationships (D'eath and Turner, 2009; Cordoni et al., 2021). If the domestication syndrome applies to domestic pigs and, again, has

led to the presence of behavioural neoteny traits, we expect aggressive levels to be lower in piglets than in wild boar hybrids at any developmental stage (*Prediction 2c*).

1.2 Materials and Methods

1.2.1. The Study Group

The research was carried out on three domestic piglet litters (mixed breed: Large White x Parma Black) and three wild boar hybrid litters (Large White breed × wild boar) at the “Ethical Farm Parva Domus”, in Cavagnolo, Turin (Italy). The study was conducted from June to December 2018 and included: (i) 24 piglets (11 females, 13 males; **Table 1**), with different mothers and the same father (Parma Black); and (ii) 27 wild boar hybrids (13 females, 14 males; **Table 1**) with different mothers and same father (one wild boar captured a few days after mating with sows). All sows were housed in spaces of grassland– woodland natural habitat, and during the observation period all the sows stayed isolated with their litters either with a physical barrier (fence, for the piglet mothers) or by providing food in a separate space (≥ 100 m²). This condition was already present and part of the farmer management and not changed for the study purposes. Sows received food pellets (Ciclo Unico P, SILDAMIN®) each morning between 8:30 a.m. and 10:30 a.m. Piglets and wild boar hybrids were able to opportunistically integrate maternal milk with roots, leaves, and fruits found in the natural environment or with pellets left by their mother. Piglet tails and teeth were kept intact, and males (of both hybrids and piglets) were castrated. The animals followed the natural day/night cycle and did not show any aberrant or stereotypical behaviours. To facilitate the identification, each individual had a unique marking with spray Raidex® for livestock; the marking was renewed every 4–7 days depending on weather conditions. During the video recording, the observers stayed around 10 m from piglets and sows.

Table 1. Composition of the litters of piglets and wild boar hybrids.

Category	Litter	Individuals	Date of birth
Piglets	1	11 (5 female; 6 male)	16 Sept 2018
	2	6 (2 female; 4 male)	3 Oct 2018
	3	8 (4 female; 4 male)	5 Nov 2018
Wild boar hybrids	1	9 (5 female; 4 male)	14 Jun 2018
	2	8 (5 female; 3 male)	05 Jun 2018
	3	10 (3 female; 7 male)	10 Jun 2018

1.2.2 Data Collection and Video Analyses

Owing to the spatial separation of sows and their litters from others, all observed interactions occurred within litters. The behavioural patterns were video-recorded from Jun–Jul 2018 for hybrids and Sept–Dec 2018 for piglets by using HD/Full HD Sony HDR- XR200 and Panasonic HC-W3580 cameras. We collected and analysed 15.92

h (Mean $\pm$ SE: 5.31 h $\pm$ 0.60) and 12.05 h (Mean $\pm$ SE: 4.02 h $\pm$ 0.46) for piglets and wild boar hybrids, respectively. During the first week after birth, all subjects were habituated to the presence of the observers and data collection started around the end of the first week of life. Via the all occurrences sampling method (Altmann, 1974), we recorded social play (n = 477) and aggression (n = 166) sessions in both piglets and hybrids (**Figure 1**).



Figure 1. Wild boar hybrids (left) and piglets (right) during lactation (piglets are marked for individual identification).

Prior to starting the video analysis, video coders (M.G., Ed.C., M.C.) were supervised by G.C. and I.N. in behavioural coding and the video analysis started when the interobserver reliability scores measured via Cohen's k reached 0.83. The interobserver reliability between video coders was calculated using the R function 'cohen.cappa' and libraries 'irr' and 'psych' (R version 3.5.3). The videos were analysed frame by frame or slow motion via freeware software VLC 2.2.1 (jump-to-time extension). For each interaction session, we extracted from videos the following information: (1) the identity of the subjects involved, (2) individual features (sex, age), (3) behavioural patterns performed (Table 2), (4) time of each pattern and the length of the session(s). Finally, we defined three periods spanning 14 days of piglet life from the 6th to the 50th day of life: T₁ (6–20 days); T₂ (21–35 days); and T₃ (36–50 days).

Table 2. Ethogram with play patterns recorded in the current study

Play patterns			
Category	Behavioural pattern	Type	Description
Offensive	Attempt play bite	C	A piglet attempts to bite the partner, but there is no contact with it
	Head play knocking	C	A piglet hits another individual with the head
	Play bite	C	A piglets bites a partner by delicately closing mouth over the other's flesh
	Play lifting	C	A piglet attempts to displace a partner by lifting or levering it with snout or head
	Play mount/climb	C	A piglet places both front hoofs on the back of another piglet or sow
	Play push	C	A piglet drives its head, neck or shoulders with minimal or moderate force into another piglet's body. Occasionally, this pattern results in the displacement of the target animal. It is significantly more intensive than nudging
	Play run	LA	A piglet runs and hops in forward motions within the pen environment. Run can be performed both in solitary and social manner
Neutral	Flopping	LA	A piglet drops to the pen floor from a normal upright position to a sitting or lying position. There is no contact with an object or another individual that could cause the change in position
	Head tossing	LA	A piglet gently head shakes from one side to another
	Hopping	LA	A piglet has either its two front feet or all four feet off the pen floor at one time through an energetic upwards jumping movement. The piglet continues facing the same original direction for the whole of the behaviour
	Leg spreading	LA	A piglet spreads its fore and hind limbs and it moves quickly from side to side
	Nudge	C	A piglet uses its snout to gently touch another piglet's body (excluding nose-nose contact). It is more intensive than touching, but also more gentle than pushing
	Object play	LA	A piglet manipulates an item or securely holds it in its mouth, energetically shaking it or carrying it around the pen
	Pivot	LA	A piglet twirls its body on the horizontal plane by a minimum of 90°. Pivot is usually associated with jumping on the spot
	Scamper	LA	A piglet performs two or more forward directed hops in quick succession of each other usually associated with excitability

Defensive	Play kneeling	LA	A piglet goes down on its knees while playing
	Play lying down	LA	A piglet places itself in a horizontal position during play
	Play sitting	LA	A piglet sits during play

Integrated or modified from other ethograms (Horback, 2014; Newberry and Wood-Gush, 1988; Newberry et al., 1988; Rauw, 2013; Špinka, 2017; Rushen and Pajor, 1987; O'Malley et al., 2022). LA = locomotor/acrobatic pattern, C = contact pattern.

Table 3. Ethogram with aggressive patterns recorded in the current study

Aggressive patterns			
Category	Behavioural pattern	Type	Description
Offensive	Aggressive bite	C	A piglet opens its mouth and closes its teeth tight on a small piece of the opponent's flesh (except tail)
	Aggressive head knocking	C	A piglet lunges or jerks its head with physical contact and mouth closed
	Aggressive kick	C	A piglet kicks with one or both hind limbs the opponent, striking it
	Aggressive lifting	C	A piglet attempts to displace the opponent by lifting or levering it with snout or head
	Aggressive mount/climb	C	A piglet forces the opponent to move away by rising upon the rear of the partner
	Aggressive push	C	A piglet presses its head, neck, shoulder or body against the opponent in an aggressive context
	Attempt aggressive bite	C	A piglet opens its mouth, directs or turns its head towards the body of the opponent and closes its mouth without contact
Neutral	Head tilting	LA	A piglet moves the head to the side when the opponent passes or gets closer
	Threat	LA	A piglet arches the back to the opponent or makes a forward movement of the head and stares at the opponent with no physical contact
	Rest during fight	LA	A piglet rests and does not exhibit aggressive behavior while being hit – during a reciprocal real fight sessions - for at least 3 seconds. (reciprocal fighting must occur before and after this event for it to be classified as a rest during fight).
Defensive	Asymmetric parallel	C	The piglets involved in a real fight are facing in the same direction, standing side by side and one of them is slightly ahead of the other. A piglet - the one placed slightly in front of the other - moves forward, pushing the opponent away with his shoulder and moving his head away from the opponent to avoid having its ears bitten.
	Avoidance	LA	A piglet moves away with a depressed tail when the opponent approaches

	Flee	LA	A piglet runs away from the opponent. The opponent can react with a chase.
	Withdrawal	LA	A piglet tries to leave a reciprocal real fight session, the opponent continues to bite the recipient with a rate greater than one bite for 3s, and the recipient reacts with any harmful aggression for more than 3s. After that, the piglets involved do not have interactions for at least 3s.

Integrated or modified from other ethograms (Horback, 2014; Newberry and Wood-Gush, 1988; Newberry et al., 1988; Rauw, 2013; Špinka, 2017; Rushen and Pajor, 1987; O'Malley et al., 2022). LA = locomotor/acrobatic pattern, C = contact pattern.

1.2.3 Operational Definitions and Structural Indices

We considered a play session as started when a piglet directed any playful pattern (see Table 2) towards the littermate and finished when both players stopped the interaction, with one of them moving away or with a third subject interrupting the session. Play sessions were considered as different if the play interaction stopped for at least 10s (Cordoni et al., 2021). We considered an aggression to have started when a piglet directed any aggressive pattern (see Table 3) towards a litter-mate and it ended with one of the opponents moving or fleeing away. Two aggressive sessions were considered as different if the real fight interaction stopped for at least 10s (Cordoni et al., 2021). The duration and frequency of social play and aggressive events were calculated considering all sessions.

For the purpose of this study, play fights and real fights included at least two behavioural patterns listed in the ethogram (Horback, 2014; Newberry and Wood-Gush, 1988; Newberry et al., 1988; Rauw, 2013; Špinka, 2017; Rushen and Pajor, 1987; O'Malley et al., 2022), Tables 2 and 3). For both play and real fights, the patterns were classified as Offensive (O, unidirectional patterns performed to threat, attack, pursue, and injure the opponent), Defensive/Submissive (D/S, patterns of body protection, contact avoidance, and submission), and Neutral (N, neither offensive nor defensive; play fight - Table 2; real fight - Table 3). For each play fight and real fight session, we calculated different indices in order to evaluate the distribution and variability of the motor patterns performed within the session and the symmetry of the interaction. In particular, we considered the indices listed below.

Asymmetry Index (AI): The play Asymmetry Index (pAI; (Cordoni et al., 2016, 2018, 2021) was used to quantify the level of play- fighting asymmetry. For each interaction we calculated pAI as follows: 'the number of offensive patterns by A towards B *plus* the number of defensive patterns by B towards A' *minus* 'the number of offensive patterns by B towards A *plus* the number of defensive patterns by A towards B' divided by 'the total number of patterns performed by both playmates'. The formula of pAI is reported below:

$$pAI = \frac{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) - (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B})}{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) + (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B}) + \text{neutral}_{A+B}}$$

Based on pAI, we also calculated the aggression Asymmetry Index (aAI) to quantify the level of real fight asymmetry. For each interaction we calculated aAI as follows: ‘the number of offensive patterns by A towards B *plus* the number of defensive patterns by B towards A’ *minus* ‘the number of offensive patterns by B towards A *plus* the number of defensive patterns by A towards B’ divided by ‘the total number of patterns performed by both opponents’. The formula of aAI is reported below:

$$aAI = \frac{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) - (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B})}{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) + (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B}) + \text{neutral}_{A+B}}$$

Both pAI and aAI range from -1 to +1 with main values indicating (i) a complete symmetry of the session (zero), (ii) a complete asymmetry of the session in favour of A (+1), and (iii) a complete asymmetry of the session in favour of B (-1).

The indices listed below were adapted from indices employed for measuring biodiversity in ecological studies (Türkmen and Kazancı, 2010; Morris, 2014; Supriatna, 2018). In particular, we considered each play/real fight session as an ‘ecosystem’, including individuals (i.e., in our case all the behavioural patterns included in the session) belonging to different species (i.e., in our case the different types of behavioural patterns included in the session). Based on this approach, we calculated the Shannon and the Pielou indices.

Shannon index (H’; also known as Shannon’s diversity index, Shannon–Wiener index, Shannon–Weaver index, and Shannon entropy): it is the most common diversity index used in ecological studies and it allows the description of both richness and evenness of a specific ecosystem [71–73] (Shannon, 1949; Mandaville, 2002; Keylock, 2005). Evenness refers to the relative abundance of species, quantifying the equality of a community, and its value is higher if each species has equal distribution in an ecosystem [74–76] (Wilsey and Potvin, 2000; Eliazar and Sokolov, 2012; Daly et al., 2018). The mathematical formula of the Shannon index is:

$$H' = -\sum[(n_i/N) * (\ln n_i/N)]$$

In particular, n_i is the number of individuals belonging to the species i and N is the total number of individuals in a specific ecosystem. H' values are generally between zero and five; when they are equal or higher than four indicate a great level of biodiversity and a balanced ecosystem structure. In our study, n_i is represented by numbers of patterns belonging to the type i , and N is represented by the total number of patterns

composing a session. A high value of H' indicates a great behavioural pattern variability in terms of different types of patterns performed in a single session.

Pielou index (J; also known as Species evenness): it derives from Shannon index and is the measure of the distribution of individuals among species within a specific ecosystem (Pielou, 1966). The mathematical formula of *Pielou index* is:

$$J = \frac{H'}{H'_{\max}}$$

H' is the observed value of Shannon index, and $H'_{\max}$ is the $\ln S$ with S representing the total number of species. The values of J vary between zero and one; when they are close to one it means that individuals are even distributed among species (Pielou, 1966). In the present study S is represented by the total number of different types of behavioural patterns. To calculate the indices and run analyses on them we selected the dyads with at least two sessions of either play or real fighting.

1.2.4 Statistical Elaboration

Owing to non-normal data distribution (Kolmogorov–Smirnov, $24 \leq n \leq 27$, $0.000 < p \leq 0.045$) we applied non-parametric tests at the individual level. In particular we applied the Mann–Whitney test for two independent samples (Siegel and Castellan, 1988) to compare the mean of social play and aggression frequency and duration between piglets and wild boar–pig hybrids. We applied the Friedman’s test for k dependent samples to compare social play and aggression session levels across periods. We applied the Dunn post hoc test for pairwise comparisons, with the significance level of probability adjusted downward using the Bonferroni correction.

Owing to normal data distribution (Kolmogorov–Smirnov, $10 \leq n \leq 58$; $0.051 \leq p \leq 0.200$) at the dyadic level, we applied the parametric t -test to independent samples in order to compare pAI/aAI values and H' and J values between piglets and wild boar hybrids for either play fight and real fight sessions. Due to possible data pseudoreplication (same individuals included in different dyads), we applied a resampling procedure (bootstrapping, 10,000 permutations). All tests were carried out via SPSS 20.0. The significance level was set at $p < 0.05$. A trend of significance was discussed for $0.05 \leq p < 0.1$.

1.3 Results

1.3.1 Prediction 1: Social Play Levels and Structure in Piglets and Wild Boar Hybrids

We found a trend of significance in the comparison between the mean frequency of social play sessions between piglets and wild boar hybrids (Mann–Whitney exact test: $U = 601.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.057$; **Figure 2a**).

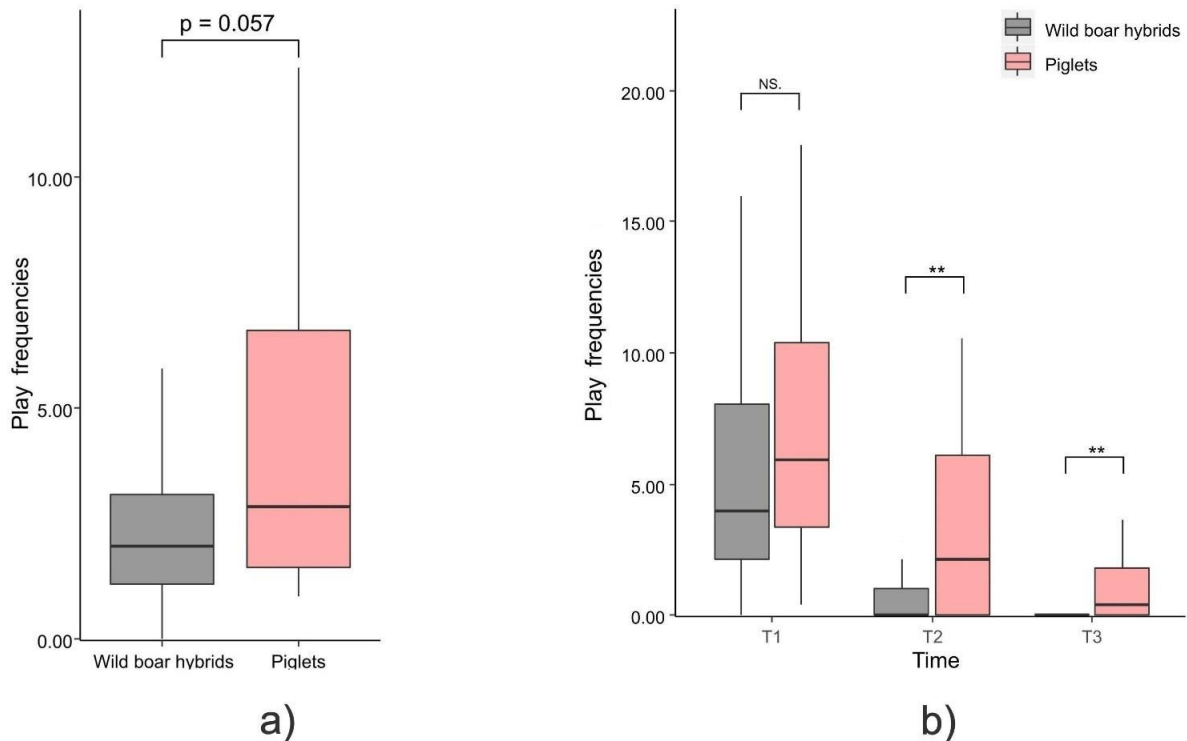


Figure 2. Box plot showing that: (a) the overall social play frequency tends to be higher in piglets than wild boar hybrids for the whole period (Mann–Whitney exact test; $p = 0.057$); (b) social play hourly frequencies (age periods: $T_1 = 6\text{--}20$ days, $T_2 = 21\text{--}35$ days, $T_3 = 36\text{--}50$ days) are higher in piglets in T_2 (Mann–Whitney exact test; $p = 0.006$) and in T_3 (Mann–Whitney exact test; $p = 0.001$). There was a significant decrease in the social play levels across periods for both hybrids and piglets (Friedman's tests, $p < 0.05$) and particularly between T_1 and T_2/T_3 (Dunn's tests: $p < 0.05$). Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, ** = $p < 0.01$.

Temporal distribution of social play. There was a significant decrease of the social play levels across periods (**Figure 2b**) and particularly from T_1 to T_2/T_3 (but not between T_2 and T_3) for both wild boar hybrids (Friedman test: $N_{\text{hybrids}} = 27$, $\chi^2 = 34.449$, $df = 2$, $p < 0.001$; and Dunn test, T_2 vs. T_1 : $Q = 3.878$, $p < 0.001$; T_3 vs. T_1 : $Q = 5.103$, $p < 0.001$; T_3 vs. T_2 : $Q = 1.225$, $p = 0.662$) and piglets (Friedman test: $n_{\text{pigs}} = 24$, $\chi^2 = 15.404$, $df = 2$, $p < 0.001$; and Dunn test, T_2 vs. T_1 : $Q = 2.742$, $p = 0.018$; T_3 vs. T_1 : $Q = 3.753$, $p = 0.001$; T_3 vs. T_2 : $Q = 1.010$, $p = 0.937$). The comparison between piglets and wild boar hybrids within each development period (T_1 , T_2 , T_3) revealed that piglets showed higher frequencies of social play sessions than wild boar hybrids in T_2 (Mann–Whitney exact test: $U = 563.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.006$; **Figure 2**) and in T_3 (Mann–Whitney exact test: $U = 549.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.001$; **Figure 2**), whereas no difference was detected in T_1 (Mann–Whitney exact test: $U = 647.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.299$; **Figure 2b**).

Structure of social play. Social play sessions were shorter in piglets than in wild boar hybrids (Mann–Whitney exact test: $U = 392.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p < 0.001$; **Figure 3a**). The asymmetry of play fight sessions (pAI) was comparable between piglets than wild boar hybrids (t -test for independent samples, $n_{\text{pig_dyads}} = 58$; $n_{\text{hybrid_dyads}} = 21$; $t = -0.318$; $p = 0.753$; **Figure 3b**). The level of pattern variability (Shannon Index, H') and evenness (Pielou Index, J) were significantly lower in piglets than in wild boar hybrids (t -test for independent samples, $n_{\text{pig_dyads}} = 58$; $n_{\text{hybrid_dyads}} = 21$; H' : $t = 3.899$; $p < 0.001$; **Figure 3c**; J : $t = 4.953$ $p < 0.001$, **Figure 3d**).

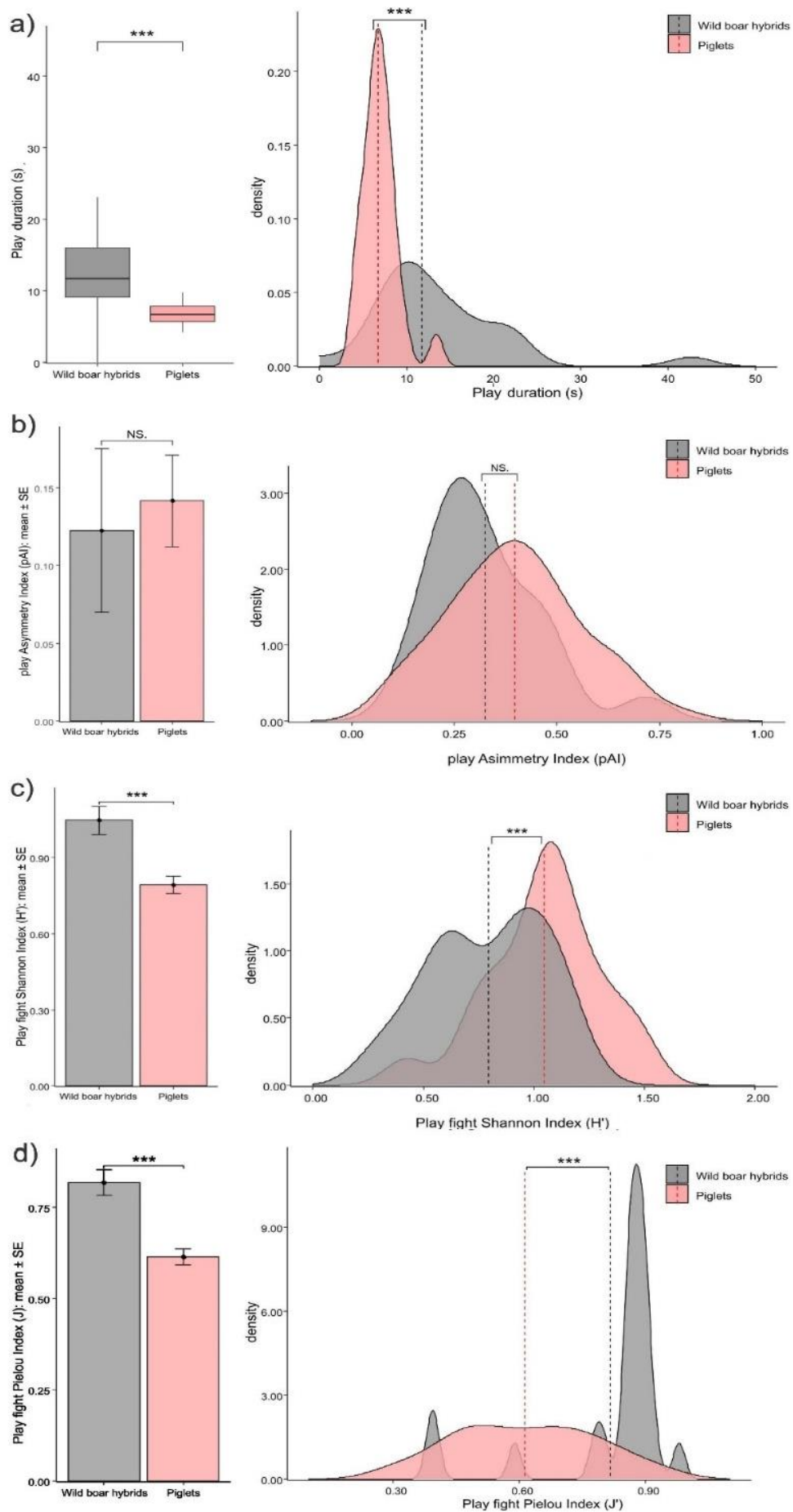


Figure 3. Differences in the duration of the play sessions between piglets and wild boar hybrids shown as bar plot (left) and density plot (right). **(a)** Piglets show shorter play sessions (Mann–Whitney exact test; $p < 0.001$). Density plot: vertical, dashed lines indicate median value. Bar plot: horizontal line: median value; box: interquartile range, vertical line: minimum and maximum values in the data. Differences in play fighting between piglets and wild boar hybrids are shown as error bar plot (left) and density plot (right). **(b)** Asymmetry Index (pAI) values; piglets and wild boar hybrids show comparable levels of asymmetry (t-test for independent samples; $p = 0.753$). **(c)** Shannon Index (H') values; piglets show the lowest levels of play fight variability (t-test for independent samples; $p < 0.001$). **(d)** Pielou Index (J) values; piglets show the lowest levels of evenness in play fight (evenness) (t-test for independent samples; $p < 0.001$). Density plot: vertical, dashed lines indicate mean value. Density plot: vertical, dashed lines indicate mean value. Error bar plot: vertical bars; Standard Error (SE) around the mean (circles). NS = non-significant, *** = $p < 0.001$.

1.3.2 Prediction 2: Aggression Level and Structure in Piglets vs. Wild Boar Hybrids

The mean frequency of aggressive sessions was significantly higher in piglets than in wild boar hybrids (Mann–Whitney exact test: $U = 574.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.017$; **Figure 4a**).

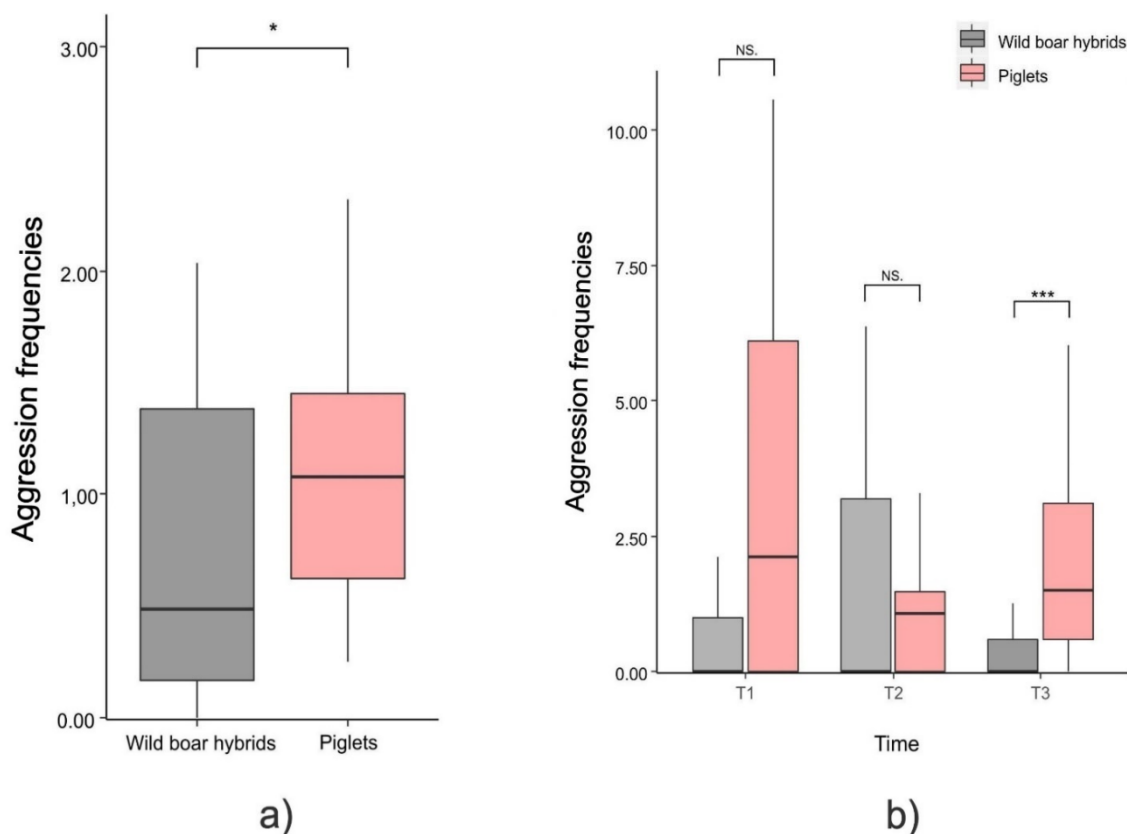


Figure 4. Box plot showing that: **(a)** the overall frequency of aggression are significantly higher than in piglets lower wild boar hybrids for the whole period (Mann–Whitney exact test; $p = 0.017$); **(b)** aggression hourly frequencies (age periods: $T_1 = 6–20$ days, $T_2 = 21–35$ days, $T_3 = 36–50$ days) are higher in piglets in T_3 (Mann–Whitney exact test; $p < 0.001$) but not in the other periods. No variation was observed across periods ($T_1–T_3$) in both hybrids and piglets (Friedman's test; $p = ns$). Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, * = $p < 0.05$, *** = $p < 0.001$.

Temporal distribution of aggression. There was no variation in the levels of aggression across development periods (T1–T3) for both wild boar hybrids (Friedman test: $n_{\text{hybrids}} = 27$, $\chi^2 = 2.282$, $df = 2$, $p = 0.320$) and piglets (Friedman test: $n_{\text{pigs}} = 24$, $\chi^2 = 3.376$, $df = 2$, $p = 0.185$). The comparison between piglets and wild boar hybrids within each development period (T1, T2, T3) revealed that piglets showed higher frequencies of real fight sessions in T3 (Mann–Whitney exact test: $U = 514.50$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p < 0.001$; **Figure 4**), whereas no difference was detected in T1 (Mann–Whitney exact test: $U = 605.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.716$; **Figure 4**) and in T2 (Mann–Whitney exact test: $U = 624.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.114$; **Figure 4b**).

Structure of aggression. There was no significant difference in the aggression duration between piglets and wild boar hybrids (Mann–Whitney exact test: $U = 591.00$, $n_{\text{pigs}} = 24$, $n_{\text{hybrids}} = 27$, $p = 0.533$; **Figure 5a**). The asymmetry (aAI) of real fight sessions was higher in piglets than in wild boar hybrids (t-test for independent samples, $n_{\text{pig_dyads}} = 10$; $n_{\text{hybrid_dyads}} = 12$; $t = -3.062$; $p = 0.007$; **Figure 5b**). The level of pattern variability (H') was significantly lower in piglets than in wild boar hybrids (t-test for independent samples, $n_{\text{pig_dyads}} = 10$; $n_{\text{hybrid_dyads}} = 12$; $t = 2.384$; $p = 0.027$; **Figure 5c**), whereas the levels of pattern evenness (J) was comparable between piglets and wild boar hybrids (t-test for independent samples, $n_{\text{pig_dyads}} = 10$; $n_{\text{hybrid_dyads}} = 12$; $t = 1.232$; $p = 0.239$; **Figure 5d**).

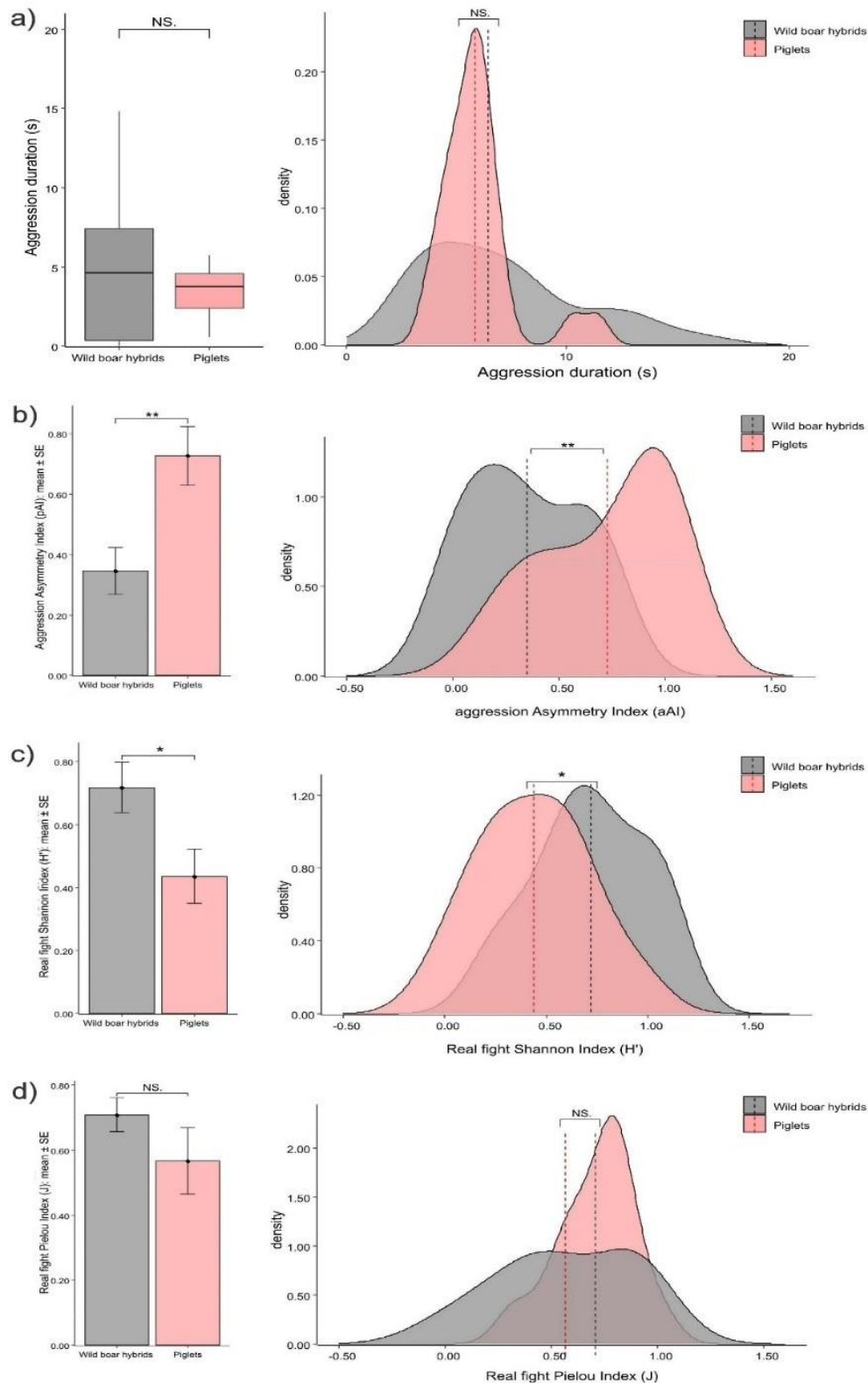


Figure 5. Differences in aggression duration between piglets and wild boar hybrids shown as bar plot (left) and density plot (right). **(a)** Piglets and wild boar hybrids show comparable levels in the aggression duration (Mann–Whitney exact test; $p = 0.539$) Density plot: vertical, dashed lines indicate median value. Bar plot: horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. Difference in real fighting between piglets and wild boar hybrids shown as error bar plot (left) and density plot (right). **(b)** Asymmetry Index (aAI) values; piglets show top levels of asymmetry (t -test for independent samples; $p = 0.007$). **(c)** Shannon Index (H') values; piglets show the lowest

levels of real fight variability (t -test for independent samples; $p = 0.027$). (d) Pielou Index (J) values; piglets and wild boar hybrids show comparable levels of real fighting in the Pielou Index (evenness) (t -test for independent samples; $p = 0.239$). Density plot: vertical, dashed lines indicate mean value. Bar plot: vertical bars. Standard Error (SE) around the mean (circles). NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$.

1.4 Discussion

1.4.1 Social Play Levels and Structure in Piglets and Wild Boar Hybrids

In this study we found that overall social play frequencies tended to be higher in piglets and wild boar hybrids (even though full significance was not reached; *Prediction 1a partly supported*; **Figure 2a**) and piglets engaged significantly more in social play than wild boar hybrids after the 20th day of life (T2 and T3; *Prediction 1c supported*; **Figure 2b**). Clearly, considering wild boar hybrids and not 100% wild boars (with both wild boar parents) can have dampened the behavioural differences between the domestic and wild form. However, taken together, the above results indicate that the domestication syndrome expectations on social play levels were partly met. The fact that the frequency of social play is comparable in the first phase of development between piglets and wild boar hybrids (within the first 20 days of life, T1) is in line with the function of this behaviour. From the very beginning, social play is important to develop the physical and social skills that are necessary to enhance self and social assessment, conflict resolution, individual recognition, group cohesion, and social bonds (Cordoni et al., 2021; Horback, 2014; Newberry and Wood-Gush, 1988; Clouard et al., 2022; Špinka, 2017; Paquette, 1994). However, in the litters under study, after the first developmental stage, play became highest in the more domestic form. One aspect of the domestication syndrome is neoteny, a slowing down in the developmental rate, which operates throughout all ontogenetic phases (Mckinney, 1998; Parker and Mckinney, 1999). Play - in particular, its persistence during individual development - is considered one of the behavioural traits highlighting the neotenic nature of a species (Coppinger et al., 2010; Morey, 1994; Palagi and Cordoni, 2012; Pellis and Pellis, 2009). Accordingly, our results can support the neotenous feature of the domestic pig compared to its wild counterpart, since play fighting decreased over time proportionally less in piglets than in wild boar hybrids (**Figure 2b**).

Increased play levels have been observed also in other domesticated species. For example, the behavioural profile of the domestic dog seems to be broadly mappable onto that of wolves, although this overlapping appears less complete when considering the propensity to play (Abrantes, 2004; Bekoff, 1972). Even if both domestic dogs and grey wolves intensively play to establish and develop social ties, a playful propensity appears to be more intense in dogs (Cordoni, 2009; Cordoni and Palagi, 2016; Smuts, 2014). In primates, bonobos - which are considered as a self-domesticated species compared to chimpanzees - show higher levels of social play than chimpanzees (Palagi and Cordoni, 2012; Hare, 2017; Hare et al., 2017; Wrangham, 2018). Contrary to our expectation (*Prediction 1b not supported*), social play sessions in piglets were shorter and, with respect to play fighting, less uniform (J) and variable (H') than in wild

boar hybrids (**Figure 3a–c**). Moreover, the level of asymmetry (pAI) of play fight sessions was comparable (**Figure 3a**) between piglets and wild boar hybrids. Overall, these results suggest that piglet players were not able to engage in more successful play sessions (as per Bekoff, 2001) than wild boar hybrid players. Indeed, successful players should be more capable of balancing their offensive and defensive patterns, thus avoiding escalation into real aggression and ultimately prolonging play (Aldis, 2013), Pellis and Pellis, 1998, 2009, 2017). The fact that this ‘balancing ability’ is not so pronounced in the more domestic form may be related to the strong competitive nature of play fight in piglets, which can be used as a substitute for aggression and may possess some features of real fighting (e.g., rank determination Cordoni et al., 2021). In *Canis lupus*, both domestic dogs and wolves can show asymmetrical play fighting behaviours because, for example, subordinates escalate play fights to try to unseat dominant animals or to probe another’s physical abilities (Marshall-Pescini et al., 2019). In this case, the competitive nature of play fight might also play a role in determining structural similarities between the wild and the domestic form.

To our knowledge, the present literature does not allow the further elaboration on this aspect due to the lack of studies that directly compare domestic and wild forms with respect to social play structure.

1.4.2 Aggression Level and Structure in Piglets vs. Wild Boar Hybrids

Contrary to what we expected, the frequency of aggression was higher in piglets than in wild boar hybrids (*Prediction 2a not supported*; **Figure 4a**). The temporal analysis revealed that aggression levels were comparable in the first 35 days of life (T1 and T2) but became higher in piglets than in wild boar hybrids after the fifth week of life (T3) (*Prediction 2c not supported*; **Figure 4b**). These results apparently contradict what is expected from the domestication syndrome, according to which aggression levels should be lower in the domestic forms and lead to increased docility (Gepts and Papa, 2002; Wilkins et al., 2014; Wright, 2015). However, at a closer look, this may not be a correct interpretation. The traits of the domestication syndrome (increased docility and decreased aggressiveness) probably apply more to the relationship between animals and humans than to the relationship between conspecifics. This is in line with the definition of domestication provided by Pride and Hongo (2020), who considers the traits of domesticated animals as the adaptation to the selection pressures of living within anthropogenic niches defined by management. Accordingly, studies on dogs have shown that domestication has been able to influence their social behaviour towards humans, with whom they establish preferential social bonds (Marshall-Pescini et al., 2019). Dogs show lower levels of aggression and higher levels of avoidance towards humans than wolves in the same conditions (Gácsi et al., 2005), and a higher tendency to seek human social contact (Frank and Frank, 1982). Thus, by reducing fear (Scott and Fuller, 1965) and increasing docility to humans (Lazzaroni et al., 2020; Range et al., 2019), domestication has shaped specific behavioural traits that favour the formation of stable bonds between domestic animals and humans. In addition, dogs can show avoidance towards conspecifics in order to

avoid conflicts, rather than behaviours of reconciliation following aggression (Cafazzo et al., 2018) as opposed to wolves, in whom frequent reconciliation behaviour has been observed (Cordoni and Palagi, 2008; Gabor et al., 1999). Consistently, Hansen Wheat et al. (2019) showed deviations between ancient and modern dog breeds for (among others) persistent aggressiveness, and they suggested that behavioural correlations related to domestication can be decoupled, leading to the maintenance of certain behavioural traits and the loss of others. Similarly, in *Sus scrofa*, different factors could have influenced aspects of domestication by shaping behavioural traits that vary depending on whether the interaction is with humans or conspecifics. The higher levels of aggression in the domestic pig may be the result of an increased competitiveness developed over the course of the domestication process as a possible by-product of the long-term cohabitation in rearing and confined spaces with patchy resources and no possible avoidance (D'eath and Turner, 2009; Newberry and Wood-Gush, 1988; Newberry and Wood-Gush, 1985). On the other hand, wild boars form groups that can frequently split into different subgroups, varying temporally in composition in relation to demographic and ecological factors. This situation may have resulted in a less stable and cohesive society compared to that of the domestic pig (D'eath and Turner, 2009; Špinka, 2017; Gabor et al., 1999; Kaminski et al., 2005).

Similar constraints (forced cohabitation for a long time, space, and resources) may also explain why - compared to wild boar hybrids - piglets showed comparable aggression duration (**Figure 5a**) and more asymmetrical (aAI; **Figure 5b**) and predictable (less variable as per H'; **Figure 5c**) real fight sessions (*Prediction 2b not supported*). Asymmetrical agonistic interactions are crucial to acquire a dominant status within social groups, as the hierarchical rank of an individual increases as the result of repeated aggressive encounters that are consistently won by that individual (Kendall, 1948; Landau, 1951; Appleby, 1983; De Vries, 1995; Drews, 1993). The dominance status deriving from such directional aggressive encounters allows high ranking individuals to access resources with priority, thus increasing their fitness (Clutton-Brock, 1982; Preuschoft and Van Schaik, 2000; Andersen et al., 2004; Sapolsky, 2005; Krebs and Davies, 2009; Norring et al., 2017). As it occurs for social play, it is not possible to go deeper in the discussion of the differences in the structure of agonistic interactions due to the lack of studies that directly compare domestic and wild forms in this respect.

A main limit of this study is that we could not compare the domestic pigs with 'full' wild boar subjects, which may have dampened some differences between the domestic and wild form. A comparison between the domestic and wild form with a larger number of individuals and more than three litters, within a single season and with the extension of data collection after weaning could help gain more information about the behavioural differences that may have emerged as a result of domestication. In particular, data on various groups, also including different breeds and extensive farming conditions, would allow further inferences on domestication syndrome at the population level and allow the distinction between traits that have been preserved by the domestication processes and more 'volatile' traits that are contingent on farming

strategies. Some of these limitations are compensated for by the quite unique opportunity to collect video data in adjacent months on wild boar hybrids (whose birth was unplanned) and piglets that were (i) raised under the same (non-intensive) environmental setting; (ii) exposed to the same management conditions (including the type and timing of food provisioning); (iii) characterized by the same level of kinship across either wild boar hybrid and piglet litters and (iv) probably minimally affected by experience given their very young age. Moreover, our results were obtained by an in-depth behavioural video analysis on an extensive session sample (more than 600 sessions involving 51 individuals).

In conclusion, our study sheds light on an interesting anthropological question, which is how artificial selection operated by *Homo sapiens* since Neolithic might have operated on behaviour to favour the transition from the wild to the domestic forms, namely *Sus scrofa*. Our results show that certain domestication traits are already present in the early stages of life but also that the domestication syndrome does not fully apply to either the playful or the aggressive domain. This mismatch is probably apparent and not actual, in that artificial selection in domestic pigs - as well as in other species - has probably produced greater tameness towards humans rather than towards conspecifics. This aspect has not been covered by this study and deserves further investigation in the future.

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Ethical Statement: This research was purely observational and non-invasive; therefore, it did not require any specific permission. Piglets were not removed from their group during observations, and they were free to perform their ordinary maintenance and social activities and to stay out of our sight if wanted.

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1.5 References

- Abrantes, R. (2004). *Evolution of canine social behavior*. Dogwise Publishing.
- Aldis, O. (2013). *Play fighting*. Elsevier Science.
- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49(3–4), 227–266. <https://doi.org/10.1163/156853974x00534>
- Andersen, L. B., Hasselstrøm, H., Grønfeldt, V., Hansen, S. E., & Karsten, F. (2004). The relationship between physical fitness and clustered risk, and tracking of clustered risk from adolescence to young adulthood: Eight years follow-up in the Danish Youth and Sport Study. *The International Journal of Behavioral Nutrition and Physical Activity*, 1(1). <https://doi.org/10.1186/1479-5868-1-6>
- Appleby, M. C. (1983). The probability of linearity in hierarchies. *Animal Behaviour*, 31(2), 600–608. [https://doi.org/10.1016/s0003-3472\(83\)80084-0](https://doi.org/10.1016/s0003-3472(83)80084-0)
- Bekoff, M. (2001). Social play behaviour: Cooperation, fairness, trust, and the evolution of morality. *Journal of Consciousness Studies: Controversies in Science & the Humanities*, 8(2), 81–90. Retrieved from <https://www.ingentaconnect.com/content/imp/jcs/2001/00000008/00000002/1075>
- Bekoff, M. (1972). The development of social interaction, play, and metacommunication in mammals: An ethological perspective. *The Quarterly Review of Biology*, 47(4), 412–434. <https://doi.org/10.1086/407400>
- Burghardt, G. M. (2005). *The genesis of animal play: Testing the limits*. MIT Press.
- Burghardt, G. M. (2011). *The Oxford handbook of the development of play* (A. D. Pellegrini & P. Nathan, Eds.). Oxford University Press.
- Cafazzo, S., Marshall-Pescini, S., Lazzaroni, M., Virányi, Z., & Range, F. (2018). The effect of domestication on post-conflict management: Wolves reconcile while dogs avoid each other. *Royal Society Open Science*, 5(7), 171553. <https://doi.org/10.1098/rsos.171553>
- Clouard, C., Resmond, R., Prunier, A., Tallet, C., & Merlot, E. (2022). Exploration of early social behaviors and social styles in relation to individual characteristics in suckling piglets. *Scientific Reports*, 12(1), 2318. <https://doi.org/10.1038/s41598-022-06354-w>
- Clutton-Brock, J. (1992). The process of domestication. *Mammal Review*, 22(2), 79–85. <https://doi.org/10.1111/j.1365-2907.1992.tb00122.x>
- Clutton-Brock, T. H. (1982). The functions of antlers. *Behaviour*, 79(2–4), 108–124. <https://doi.org/10.1163/156853982x00201>
- Coppinger, R., Glendinning, J., Torop, E., Matthay, C., Sutherland, M., & Smith, C. (1987). Degree of behavioral neoteny differentiates Canid polymorphs. *Ethology*, 75(2), 89–108. <https://doi.org/10.1111/j.1439-0310.1987.tb00645.x>

- Cordoni, G. (2009). Social play in captive wolves (*Canis lupus*): Not only an immature affair. *Behaviour*, 146(10), 1363–1385. <https://doi.org/10.1163/156853909x427722>
- Cordoni, G., Gioia, M., Demuru, E., & Norscia, I. (2021). The dark side of play: Play fighting as a substitute for real fighting in domestic pigs, *Sus scrofa*. *Animal Behaviour*, 175, 21–31. <https://doi.org/10.1016/j.anbehav.2021.02.016>
- Cordoni, G., Nicotra, V., & Palagi, E. (2016). Unveiling the "secret" of play in dogs (*Canis lupus familiaris*): Asymmetry and signals. *Journal of Comparative Psychology*, 130(3), 278–287. <https://doi.org/10.1037/com0000035>
- Cordoni, G., Norscia, I., Bobbio, M., & Palagi, E. (2018). Differences in play can illuminate differences in affiliation: A comparative study on chimpanzees and gorillas. *PloS One*, 13(3), e0193096. <https://doi.org/10.1371/journal.pone.0193096>
- Cordoni, G., & Palagi, E. (2008). Reconciliation in wolves (*Canis lupus*): New evidence for a comparative perspective. *Ethology*, 114(3), 298–308. <https://doi.org/10.1111/j.1439-0310.2008.01474.x>
- Cordoni, G., & Palagi, E. (2016). Aggression and hierarchical steepness inhibit social play in adult wolves. *Behaviour*, 153(6–7), 749–766. <https://doi.org/10.1163/1568539x-00003326>
- Daly, A. J., Baetens, J. M., & De Baets, B. (2018). Ecological diversity: Measuring the unmeasurable. *Mathematics*, 6(7), 119. <https://doi.org/10.3390/math6070119>
- De Boer, S. F., Buwalda, B., & Koolhaas, J. (2016). *Stress: Concepts, cognition, emotion, and behavior* (Vol. 36). Academic Press.
- de Vries, H. (1995). An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Animal Behaviour*, 50(5), 1375–1389. [https://doi.org/10.1016/0003-3472\(95\)80053-0](https://doi.org/10.1016/0003-3472(95)80053-0)
- Death, R. B., & Turner, S. P. (2009). *The welfare of pigs*. Springer.
- Del Savio, L., & Mameli, M. (2020). Human domestication and the roles of human agency in human evolution. *History and Philosophy of the Life Sciences*, 42(2), 21. <https://doi.org/10.1007/s40656-020-00315-0>
- Dellmeier, G. R., & Friend, T. H. (1991). Behavior and extensive management of domestic sows (*Sus scrofa*) and litters. *Applied Animal Behaviour Science*, 29(1–4), 327–341. [https://doi.org/10.1016/0168-1591\(91\)90258-y](https://doi.org/10.1016/0168-1591(91)90258-y)
- Drews, C. (1993). The concept and definition of dominance in animal behaviour. *Behaviour*, 125(3–4), 283–313. <https://doi.org/10.1163/156853993x00290>
- Eliazar, I. I., & Sokolov, I. M. (2012). Measuring statistical evenness: A panoramic overview. *Physica A*, 391(4), 1323–1353. <https://doi.org/10.1016/j.physa.2011.09.007>

Frank, H., & Frank, M. G. (1982). On the effects of domestication on canine social development and behavior. *Applied Animal Ethology*, 8(6), 507–525. [https://doi.org/10.1016/0304-3762\(82\)90215-2](https://doi.org/10.1016/0304-3762(82)90215-2)

Frantz, L., Meijaard, E., Gongora, J., Haile, J., Groenen, M. A. M., & Larson, G. (2016). The evolution of Suidae. *Annual Review of Animal Biosciences*, 4(1), 61–85. <https://doi.org/10.1146/annurev-animal-021815-111155>

Gabor, T. M., Hellgren, E. C., Van Den Bussche, R. A., & Silvy, N. J. (1999). Demography, sociospatial behaviour and genetics of feral pigs (*Sus scrofa*) in a semi-arid environment. *Journal of Zoology (London, England: 1987)*, 247(3), 311–322. <https://doi.org/10.1111/j.1469-7998.1999.tb00994.x>

Gácsi, M., Györi, B., Miklósi, Á., Virányi, Z., Kubinyi, E., Topál, J., & Csányi, V. (2005). Species-specific differences and similarities in the behavior of hand-raised dog and wolf pups in social situations with humans. *Developmental Psychobiology*, 47(2), 111–122. <https://doi.org/10.1002/dev.20082>

Gepts, P., & Papa, R. (2002). *Encyclopedia of Life Sciences*. Nature Publishing Group.

Goodwin, D., Bradshaw, J. W. S., & Wickens, S. M. (1997). Paedomorphosis affects agonistic visual signals of domestic dogs. *Animal Behaviour*, 53(2), 297–304. <https://doi.org/10.1006/anbe.1996.0370>

Gustafsson, M., Jensen, P., de Jonge, F. H., Illmann, G., & Spinka, M. (1999). Maternal behaviour of domestic sows and crosses between domestic sows and wild boar. *Applied Animal Behaviour Science*, 65(1), 29–42. [https://doi.org/10.1016/s0168-1591\(99\)00048-9](https://doi.org/10.1016/s0168-1591(99)00048-9)

Gustafsson, M., Jensen, P., de Jonge, F. H., & Schuurman, T. (1999). Domestication effects on foraging strategies in pigs (*Sus scrofa*). *Applied Animal Behaviour Science*, 62(4), 305–317. [https://doi.org/10.1016/s0168-1591\(98\)00236-6](https://doi.org/10.1016/s0168-1591(98)00236-6)

Hansen Wheat, C., Fitzpatrick, J. L., Rogell, B., & Temrin, H. (2019). Behavioural correlations of the domestication syndrome are decoupled in modern dog breeds. *Nature Communications*, 10(1), 2422. <https://doi.org/10.1038/s41467-019-10426-3>

Hare, B. (2017). Survival of the friendliest: *Homo sapiens* evolved via selection for prosociality. *Annual Review of Psychology*, 68(1), 155–186. <https://doi.org/10.1146/annurev-psych-010416-044201>

Hare, B., Wobber, V., & Wrangham, R. (2012). The self-domestication hypothesis: Evolution of bonobo psychology is due to selection against aggression. *Animal Behaviour*, 83(3), 573–585. <https://doi.org/10.1016/j.anbehav.2011.12.007>

Hare, B., Wrangham, R., Muller, M., & Wrangham, R. W. (2017). *Chimpanzees and Human Evolution* (R. Pilbeam, Ed.). Harvard University Press.

Himmler, B. T., Stryjek, R., Modlinska, K., Derksen, S. M., Pisula, W., & Pellis, S. M. (2013). How domestication modulates play behavior: A comparative analysis between wild rats and a

laboratory strain of *Rattus norvegicus*. *Journal of Comparative Psychology* (Washington, D.C.: 1983), 127(4), 453–464. <https://doi.org/10.1037/a0032187>

Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2(2), 186. <https://doi.org/10.12966/abc.05.08.2014>

Jensen, P. (1995). The weaning process of free-ranging domestic pigs: Within- and between-litter variations. *Ethology: Formerly Zeitschrift Für Tierpsychologie*, 100(1), 14–25. <https://doi.org/10.1111/j.1439-0310.1995.tb00311.x>

Kaminski, G., Brandt, S., Baubet, E., & Baudoin, C. (2005). Life-history patterns in female wild boars (*Sus scrofa*): Mother–daughter postweaning associations. *Canadian Journal of Zoology*, 83(3), 474–480. <https://doi.org/10.1139/z05-019>

Kendall, M. G. (1948). *Rank correlation methods*. Griffin.

Keylock, C. J. (2005). Simpson diversity and the Shannon–Wiener index as special cases of a generalized entropy. *Oikos (Copenhagen, Denmark)*, 109(1), 203–207. <https://doi.org/10.1111/j.0030-1299.2005.13735.x>

Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. A. (2013). The resident-intruder paradigm: A standardized test for aggression, violence and social stress. *Journal of Visualized Experiments: JoVE*, (77), e4367. <https://doi.org/10.3791/4367>

Krebs, J. R., & Davies, N. B. (2009). *An introduction to behavioural ecology* (3rd ed.). Wiley-Blackwell.

Künzl, C., & Sachser, N. (1999). The behavioral endocrinology of domestication: A comparison between the domestic guinea pig (*Cavia aperea f. porcellus*) and its wild ancestor, the cavy (*Cavia aperea*). *Hormones and Behavior*, 35(1), 28–37. <https://doi.org/10.1006/hbeh.1998.1493>

Kurz, J. C., & Marchinton, R. L. (1972). Radiotelemetry studies of feral hogs in South Carolina. *The Journal of Wildlife Management*, 36(4), 1240. <https://doi.org/10.2307/3799254>

Landau, H. G. (1951). On dominance relations and the structure of animal societies: I. Effect of inherent characteristics. *The Bulletin of Mathematical Biophysics*, 13(1), 1–19. <https://doi.org/10.1007/bf02478336>

Larson, G., Albarella, U., Dobney, K., Rowley-Conwy, P., Schibler, J., Tresset, A., ... Cooper, A. (2007). Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 104(39), 15276–15281. <https://doi.org/10.1073/pnas.0703411104>

Lazzaroni, M., Range, F., Backes, J., Portele, K., Scheck, K., & Marshall-Pescini, S. (2020). The effect of domestication and experience on the social interaction of dogs and wolves with a human companion. *Frontiers in Psychology*, 11, 785. <https://doi.org/10.3389/fpsyg.2020.00785>

Lega, C., Raia, P., Rook, L., & Fulgione, D. (2016). Size matters: A comparative analysis of pig domestication. *The Holocene*, 26(2), 327–332. <https://doi.org/10.1177/0959683615596842>

Leno-Coloardo, J., Hudson, N. J., Reverter, A., & Pérez-Enciso, M. (2017). A pathway-centered analysis of pig domestication and breeding in Eurasia. *G3 (Bethesda, Md.)*, 7(7), 2171–2184. <https://doi.org/10.1534/g3.117.042671>

Lord, K. A., Larson, G., Coppinger, R. P., & Karlsson, E. K. (2020). The history of Farm foxes undermines the animal domestication syndrome. *Trends in Ecology & Evolution*, 35(2), 125–136. <https://doi.org/10.1016/j.tree.2019.10.011>

Mandaville, S. M. (2002). *Benthic macroinvertebrates in freshwaters: In taxa tolerance values, metrics, and protocols* (Vol. 128). Soil & Water Conservation Society of Metro Halifax.

Marshall-Pescini, S., Schaebs, F. S., Gaugg, A., Meinert, A., Deschner, T., & Range, F. (2019). The role of oxytocin in the dog–owner relationship. *Animals: An Open Access Journal from MDPI*, 9(10), 792. <https://doi.org/10.3390/ani9100792>

McKinney, M. L. (1998). *Evolution, and development*. Psychology Press.

McNamara, K. (1997). *Shapes of time: The evolution of growth and development*. Johns Hopkins University Press.

Mendl, M. T. (1994). The social behaviour of non-lactating cows and its implications for managing sow aggression. *Pig Veterinary Journal*, 34, 9–20.

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.-M., Fisher, A. D., Hinch, G. N., ... Beaumont, C. (2005). Genetics of adaptation and domestication in livestock. *Livestock Production Science*, 93(1), 3–14. <https://doi.org/10.1016/j.livprodsci.2004.11.001>

Morey, D. F. (1994). The early evolution of the domestic dog. *American Scientist*, 82(4), 336. Retrieved from <http://www.jstor.org/stable/29775234>

Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., ... Rillig, M. C. (2014). Choosing and using diversity indices: Insights for ecological applications from the German Biodiversity Exploratories. *Ecology and Evolution*, 4(18), 3514–3524. <https://doi.org/10.1002/ece3.1155>

Murphy, M. R. (1985). History of the capture and domestication of the Syrian golden hamster (*Mesocricetus auratus* Waterhouse). In *The Hamster* (pp. 3–20). Springer US.

Newberry, R. C., Wood-Gush, D. G., & Hall, J. W. (1988). Playful behaviour of piglets. *Behavioural Processes*, 17(3), 205–216. [https://doi.org/10.1016/0376-6357\(88\)90004-6](https://doi.org/10.1016/0376-6357(88)90004-6)

Newberry, R. C., & Wood-Gush, D. G. M. (1988). Development of some behaviour patterns in piglets under semi-natural conditions. *Animal Science*, 46(1), 103–109. <https://doi.org/10.1017/s0003356100003160>

Newberry, R. C., & Wood-Gush, D. G. M. (1985). The suckling behaviour of domestic pigs in a semi-natural environment. *Behaviour*, 95(1–2), 11–25. <https://doi.org/10.1163/156853985x00028>

Norring, M., Valros, A., Bergman, P., Marchant, J. N., & Heinonen, M. (2019). Body condition, live weight, and success in agonistic encounters in mixed parity groups of sows during gestation. *Animal: An International Journal of Animal Bioscience*, 13(2), 392–398. <https://doi.org/10.1017/s1751731118001453>

Norscia, I., Palagi, E., Jolly, A., Tatterall, I., & Huffman, M. (2016). *The missing lemur link: An ancestral step in the evolution of human behaviour*. Cambridge University Press.

O'Malley, C. I., Steibel, J. P., Bates, R. O., Ernst, C. W., & Siegford, J. M. (2022). The social life of pigs: Changes in affiliative and agonistic behaviors following mixing. *Animals: An Open Access Journal from MDPI*, 12(2), 206. <https://doi.org/10.3390/ani12020206>

Palagi, E., & Cordoni, G. (2012). The right time to happen: Play developmental divergence in the two *Pan* species. *PloS One*, 7(12), e52767. <https://doi.org/10.1371/journal.pone.0052767>

Paquette, D. (1994). Fighting and playfighting in captive adolescent chimpanzees. *Aggressive Behavior*, 20(1), 49–65. [https://doi.org/10.1002/1098-2337\(1994\)20:1<49::aid-ab2480200107>3.0.co;2-c](https://doi.org/10.1002/1098-2337(1994)20:1<49::aid-ab2480200107>3.0.co;2-c)

Parker, S. T., & McKinney, M. L. (n.d.). *The evolution of cognitive development in monkeys, apes, and humans*. John Hopkins.

Pellis, S. M., & Pellis, V. C. (1998). Play fighting of rats in comparative perspective: A schema for neurobehavioral analyses. *Neuroscience and Biobehavioral Reviews*, 23(1), 87–101. [https://doi.org/10.1016/s0149-7634\(97\)00071-7](https://doi.org/10.1016/s0149-7634(97)00071-7)

Pellis, S. M., & Pellis, V. C. (2017). What is play fighting and what is it good for? *Learning & Behavior*, 45(4), 355–366. <https://doi.org/10.3758/s13420-017-0264-3>

Pellis, S., & Pellis, V. (2013). *The playful brain: Venturing to the limits of neuroscience*. Oneworld.

Petersen, H. V., Vestergaard, K., & Jensen, P. (1989). Integration of piglets into social groups of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 23(3), 223–236. [https://doi.org/10.1016/0168-1591\(89\)90113-5](https://doi.org/10.1016/0168-1591(89)90113-5)

Petersen, V., Recén, B., & Vestergaard, K. (1990). Behaviour of sows and piglets during farrowing under free-range conditions. *Applied Animal Behaviour Science*, 26(1–2), 169–179. [https://doi.org/10.1016/0168-1591\(90\)90096-v](https://doi.org/10.1016/0168-1591(90)90096-v)

Pielou, E. C. (1966). Species-diversity and pattern-diversity in the study of ecological succession. *Journal of Theoretical Biology*, 10(2), 370–383. [https://doi.org/10.1016/0022-5193\(66\)90133-0](https://doi.org/10.1016/0022-5193(66)90133-0)

Preuschoft, S., & Van Schaik, C. P. (2000). Dominance and communication; Nature conflict resolution. *Nature Conflict Resolution*, 77–105.

Price, E. O. (1984). Behavioral aspects of animal domestication. *The Quarterly Review of Biology*, 59(1), 1–32. <https://doi.org/10.1086/413673>

Price, E. O. (1999). Behavioral development in animals undergoing domestication. *Applied Animal Behaviour Science*, 65(3), 245–271. [https://doi.org/10.1016/s0168-1591\(99\)00087-8](https://doi.org/10.1016/s0168-1591(99)00087-8)

Price, M., & Hongo, H. (2020). The archaeology of pig domestication in Eurasia. *Journal of Archaeological Research*, 28(4), 557–615. <https://doi.org/10.1007/s10814-019-09142-9>

Range, F., Marshall-Pescini, S., Kratz, C., & Virányi, Z. (2019). Wolves lead and dogs follow, but they both cooperate with humans. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-40468-y>

Rauw, W. M. (2013). A note on the consistency of a behavioral play marker in piglets. *Journal of Animal Science and Biotechnology*, 4(1), 33. <https://doi.org/10.1186/2049-1891-4-33>

Rushen, J., & Pajor, E. (1987). Offence and defence in fights between young pigs (*Sus scrofa*). *Aggressive Behavior*, 13(6), 329–346. [https://doi.org/10.1002/1098-2337\(1987\)13:6<329::aid-ab2480130602>3.0.co;2-3](https://doi.org/10.1002/1098-2337(1987)13:6<329::aid-ab2480130602>3.0.co;2-3)

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science*, 308(5722), 648–652. <https://doi.org/10.1126/science.1106477>

Scott, J. P., & Fuller, J. L. (1965). *Genetics and the social behaviour of the dog*. University of Chicago Press.

Serpell, J. (1989). *The walking larder* (J. Clutton-Brock, Ed.). Routledge.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, 27(3), 379–423. <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>

Siegel, S., & Castellan, N. J. (1988). *Nonparametric statistics for the behavior sciences*. McGraw-Hill.

Smuts, B. (2014). *The social dog*. Academic Press.

Spinka, M., Illmann, G., de Jonge F, Andersson, M., Schuurman, T., & Jensen, P. (2000). Dimensions of maternal behaviour characteristics in domestic and wild-domestic crossbred sows. *Applied Animal Behaviour Science*, 70(2), 99–114. [https://doi.org/10.1016/s0168-1591\(00\)00151-9](https://doi.org/10.1016/s0168-1591(00)00151-9)

Spinka, M. (Ed.). (2017). *Advances in pig welfare*. Woodhead Publishing.

Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science (Penicuik, Scotland)*, 48(2), 419–425. <https://doi.org/10.1017/s0003356100040411>

Supriatna, J. (2018). Biodiversity indexes: Value and evaluation purposes. *E3S Web of Conferences*, 48, 01001. <https://doi.org/10.1051/e3sconf/20184801001>

- Tennessen, T., & Hudson, R. J. (1981). Traits relevant to the domestication of herbivores. *Applied Animal Ethology*, 7(1), 87–102. [https://doi.org/10.1016/0304-3762\(81\)90054-7](https://doi.org/10.1016/0304-3762(81)90054-7)
- Theofanopoulou, C., Gastaldon, S., O'Rourke, T., Samuels, B. D., Martins, P. T., Delogu, F., ... Boeckx, C. (2017). Self-domestication in *Homo sapiens*: Insights from comparative genomics. *PloS One*, 12(10), e0185306. <https://doi.org/10.1371/journal.pone.0185306>
- Trut, L. (1999). Early Canid domestication: The farm-fox experiment. *American Scientist*, 87(2), 160. <https://doi.org/10.1511/1999.2.160>
- Trut, L. N. (1998). The evolutionary concept of destabilizing selection: Status quo in commemoration of D. K. Belyaev. *Journal of Animal Breeding and Genetics*, 115(1–6), 415–431. <https://doi.org/10.1111/j.1439-0388.1998.tb00364.x>
- Trut, L., Oskina, I., & Kharlamova, A. (2009). Animal evolution during domestication: The domesticated fox as a model. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 31(3), 349–360. <https://doi.org/10.1002/bies.200800070>
- Türkmen, G., & Kazancı, N. (2010). Applications of various biodiversity indices to benthic macroinvertebrate assemblages in streams of a national park in Turkey. *Rev. Hydrobiol*, 3, 111–125.
- Waller, B. M., Peirce, K., Caeiro, C. C., Scheider, L., Burrows, A. M., McCune, S., & Kaminski, J. (2013). Paedomorphic facial expressions give dogs a selective advantage. *PloS One*, 8(12), e82686. <https://doi.org/10.1371/journal.pone.0082686>
- Wilkins, A. S., Wrangham, R. W., & Fitch, W. T. (2014). The “domestication syndrome” in mammals: A unified explanation based on neural crest cell behavior and genetics. *Genetics*, 197(3), 795–808. <https://doi.org/10.1534/genetics.114.165423>
- Wilsey, B. J., & Potvin, C. (2000). Biodiversity and ecosystem functioning: Importance of species evenness in an Old Field. *Ecology*, 81(4), 887–892. [https://doi.org/10.1890/0012-9658\(2000\)081\[0887:baefio\]2.0.co;2](https://doi.org/10.1890/0012-9658(2000)081[0887:baefio]2.0.co;2)
- Wrangham, R. W. (2018). Two types of aggression in human evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 115(2), 245–253. <https://doi.org/10.1073/pnas.1713611115>
- Wright, D. (2015). The genetic architecture of domestication in animals. *Bioinformatics and Biology Insights*, 9(Suppl 4), 11–20. <https://doi.org/10.4137/BBI.S28902>
- Zeder, M. A. (2012). The domestication of animals. *Journal of Anthropological Research*, 68(2), 161–190. <https://doi.org/10.3998/jar.0521004.0068.201>
- Zeder, M. A. (2015). Core questions in domestication research. *Proceedings of the National Academy of Sciences of the United States of America*, 112(11), 3191–3198. <https://doi.org/10.1073/pnas.1501711112>

Zhang, S.-J., Wang, G.-D., Ma, P., Zhang, L.-L., Yin, T.-T., Liu, Y.-H., ... Zhang, Y.-P. (2020). Genomic regions under selection in the feralization of the dingoes. *Nature Communications*, 11(1), 671. <https://doi.org/10.1038/s41467-020-14515-6>

Zipser, B., Schleking, A., Kaiser, S., & Sachser, N. (2014). Effects of domestication on biobehavioural profiles: A comparison of domestic guinea pigs and wild cavies from early to late adolescence. *Frontiers in Zoology*, 11(1), 30. <https://doi.org/10.1186/1742-9994-11-30>

Zohary, D., Tchernov, E., & Horwitz, L. K. (1998). The role of unconscious selection in the domestication of sheep and goats. *Journal of Zoology (London, England: 1987)*, 245(2), 129–135. <https://doi.org/10.1111/j.1469-7998.1998.tb00082.x>

Cordoni, G., Collarini, E., Gioia, M., & Norscia, I. (2022). Play fighting versus real fighting in piglets (*Sus scrofa*): Similar patterns, different structure. *Behavioural Processes*, 203(104778), 104778.

2. Play fighting versus real fighting in piglets (*Sus scrofa*): Similar patterns, different structure

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Abstract

When animals engage in 'non-serious' fighting (play-fighting) they 'borrow' motor patterns especially from the aggressive context. It may be difficult to distinguish play- and real-fighting. This is particularly true for piglets (*Sus scrofa*), which can use play-fighting as a substitute for aggression. To check for the structural differences between play- and real-fighting in piglets, we: i) video recorded 496 interactions from three litters (at Parva-Domus extensive ethical farm; Turin, Italy); ii) extracted (by video analyses) duration, patterns, and data on involved individuals; iii) calculated session structural/ecological indices; and iv) compared the indices (play- vs real-fighting). Compared to real fighting – play fighting was longer (informing its rewarding nature), more symmetrical (lower asymmetry index) and variable (higher Shannon index). Moreover, play-fighting showed less pattern repetition (lower Repetition Index) and was not more polyadic and evenly distributed (comparable polyadic and Pielou indices). By being longer, and more variable and symmetrical than real-fighting, play-fighting could serve some of its functions such as motor training, social assessment and training for the unexpected. However, play-fighting in piglets did not comply with all the expected play features, possibly because play is a fluid behavioural system that under certain circumstances may escalate into or replace aggression.

2.1 Introduction

Play - including social play - is a behaviour that can serve different functions depending on the species, the social dynamics, the context, the hierarchical relations, and the individual characteristics of the players (e.g. sex, age, rank, body size; Fagen, 1981; Burghardt, 2005; Pellis, Pellis, and Bell, 2010; Norscia and Palagi, 2011; Palagi, 2011). Play can enhance cognitive, physical and social skills and it can increase the ability of individuals to flexibly adjust their behavioural responses when they face unpredictable situations (Špinka et al., 2001, Pellis and Pellis, 2009, Burghardt, 2010, Trezza et al., 2010, Vanderschuren et al., 2016). Play involves and reorganizes motor patterns that are typical of other behavioural systems (Pellis, Pellis, Pelletier, and Leca, 2019). Among the different types of play (including object and solitary play, and different social play patterns; Fagen, 1981; Burghardt, 2005), play fighting (or Rough-&-Tumble, R&T) is probably the most complex in that animals physically and variably interact in close contact during play sessions (Palagi et al., 2016, Pellis and Pellis, 2017). Animals can combine different types of play during play fighting (Burghardt, 1998, Burghardt, 2005) but this form of play mostly 'borrows' and re-organizes patterns from the aggressive domain (Pellis and Pellis, 2017). Indeed, play fighting can be related to the development of aggressive behaviour and dominance relationships in different mammalian species, including humans (e.g. Takahashi and Lore, 1983; Smith and Boulton, 1990; Paquette, 1994; Blumstein et al., 2013). Play markers have evolved in many species as honest indicators that the behaviour is harmless (Graham and Burghardt, 2010). Typical play markers for example include play bows in canids and open-mouthed play faces in primates and carnivores (Bekoff, 1975, Pellis and Pellis, 1996, Cordoni et al., 2016). However, such markers are not always expressed in a consistent way and the 'non-seriousness' of the interaction may not be evident to the human observer (Palagi et al., 2007, Petrů et al., 2009, Palagi et al., 2016). Even though play fighting is normally harmless and real fighting may result in injuries (possibly associated with pain vocalizations), this is not always the case (Rushen and Pajor, 1987, Palagi and Cordoni, 2012, Pellis and Pellis, 2017). As a result, play fighting and real fighting can differ in target and attack tactics (Pellis and Pellis, 1987) but are sometimes confused due to their structural similarity (Bekoff and Allen, 1998, Bekoff, 2014).

Following previous literature describing social play structural elements in human and non-human animals (Smith, 1989, Smith, 1997, Burghardt, 2005, Bauer and Smuts, 2007, Petrů et al., 2009, Cordoni et al., 2021, Collarini et al., 2022), play fighting (compared to real fighting) may be composed by motor patterns that can be: i) incomplete, exaggerated and more variable in their form and timing and ii) more repeated (but not stereotyped); iii) possibly – but not necessarily - modified to maintain balance during the interaction. Moreover, in several social mammalian species (including humans) both play fighting and real fighting can involve more than two individuals (polyadic play and coalitionary aggression; e.g., van Schaik et al., 2004; Palagi et al., 2014; Norscia and Palagi, 2015; Cordoni and Palagi, 2016, Cassidy and McIntyre, 2016; Cordoni et al., 2018; Sugiyama et al., 2018).

To assess what structural elements differ between play fighting and real fighting, we gathered behavioural data on immature domestic pigs (*Sus scrofa*), which are particularly suitable to investigate this aspect because they show both play- and real-fighting from the first week of life, with play fighting being also used as substitute for aggression (Cordoni et al., 2021). Indeed, right after birth, piglets compete with one another to access the most productive, frontal teats and to start establishing dominance relationships (Blackshaw et al., 1997, Ruis et al., 2000, D'Eath and Turner, 2008, Horback, 2014, Schmitt et al., 2019). Even if some level of aggregation is retained in adulthood, social interactions tend to decrease - and social play to almost disappear - after sexual maturity (Petersen et al., 1989, Horback, 2014, Cordoni et al., 2021). Indeed, in the early phases of life piglets engage in play fighting sessions, almost never observed in adults (Newberry and Wood-Gush, 1988, Horback, 2014, Brown et al., 2018). Play fighting in piglets includes one or more play markers that are not present in real fighting, such as scamper, pivot, head tossing and object shaking (Newberry and Wood-Gush, 1988, Rauw, 2013, Horback, 2014; Špinka, 2017). However, the majority of patterns used by piglets during play fighting are also used during real fighting: head-to-head wrestling, hitting at each other's flanks, head knocking the playmate, pushing, lifting, and/or biting the partner (Rushen and Pajor, 1987, Newberry and Wood-Gush, 1988, Chaloupková et al., 2007, Šilerová et al., 2010, Horback, 2014; Pellis and Pellis, 2016; Brown et al., 2018; Cordoni et al., 2021). Hence, the distinction between play- and real fighting sessions in piglets may not be always obvious. To understand what structural elements differ between the two versions of fighting (non-serious vs serious) we formulated and verified the following predictions.

2.1.1. Prediction 1

Play is supposed to be rewarding for players (Burghardt, 2005, Burghardt, 2011, Pellis and Pellis, 2009, Trezza et al., 2010, Vanderschuren, 2010, Vanderschuren et al., 2016) and the facilitation of play expression can increase play duration (van Kerkhof et al., 2013). Hence - although both play fighting and real fighting can vary in their duration - we expected that a significant proportion of play fighting sessions would be longer than real fighting sessions (*Prediction 1a*).

Piglets can engage in play sessions that can be either dyadic (involving two individuals) or polyadic (involving more than two individuals) (Worsaae and Schmidt, 1980, Chaloupková et al., 2007, Brown et al., 2018). Although conflicts in pigs usually involve two individuals, gang aggression involving multiple individuals has been described in adults (Camerlink et al., 2020, Cordoni et al., 2021). Hence, we expected to observe comparable frequencies of polyadic play fighting and real fighting (*Prediction 1b*).

2.1.2 Prediction 2

In piglets, play fighting can be used in a competitive way and the balance between offensive and defensive patterns may not be maintained, as it occurs in real fighting used to establish dominance (Weller et al., 2019; Cordoni et al., 2021). Another feature that appears to structurally distinguish either play fighting or real fighting in *Sus scrofa* immature subjects is pattern distribution, which can be more or less uniformly distributed within sessions (Collarini et al., 2022).

Based on the main features of play mentioned above and the species biology (Burghardt, 2005, Burghardt, 2011, Cordoni et al., 2021, Collarini et al., 2022), we expect that – compared to real fighting - play fighting sessions in piglets would: *2a*) not necessarily be more balanced in terms of offensive/defensive pattern exchange and distribution (with no possibility to predict – based on the present knowledge - if less or equally balanced); *2b*) be more variable in terms of different types of motor patterns performed within a single session and; *2c*) include more repeated patterns.

2.2 Materials and Methods

2.2.1. Study site, subjects and data collection

The research was carried out on three domestic pig litters (Parma Black x Parma Black and Parma Black x Large White) at ethical extensive farm Parva Domus, Cavagnolo, Turin, Italy. The study included 24 piglets (11 females, 13 males; **Table 1**) with different mothers (Linda, Nina, Black Beauty) and the same father (Bob). The three sows were kept isolated (by the farmer) with their offspring until weaning, which occurred at around 8 weeks of life. Each pre-weaning enclosure measured around 100 m² and was in an area of natural habitat equipped with straw, troughs for food, water and a shed. The individuals were able to move freely throughout the enclosure and avoid conspecifics if wanted. Sows received food pellets (Ciclo Unico P, SILDAMIN®) each morning between 08:30 and 10:30 h, whereas the maternal milk represented the major food source for piglets until weaning. No food was specifically given to piglets before weaning although they could opportunistically feed on the pellets given to the sow. Piglets could supplement maternal milk with roots, leaves and fruits found in the natural environment or with pellets left by their mother. The tails and teeth of piglets were kept intact, and males were castrated during the first 3 days of life. The animals followed the natural day/night cycle and did not perform any aberrant or stereotyped behaviour.

Table 1. Composition of the litters of piglets (F: female; M: male)

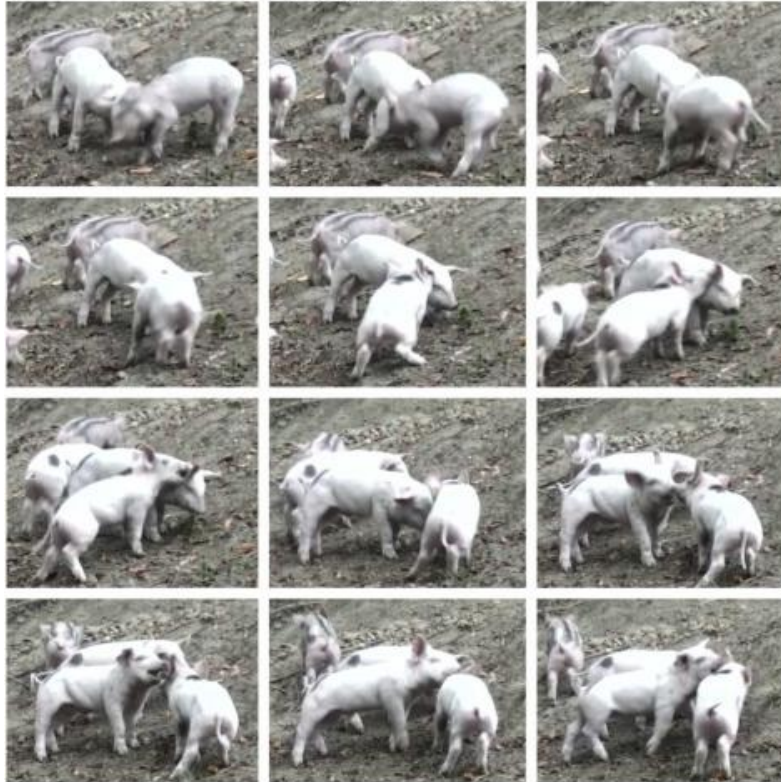
Litter	Individuals	Date of birth
LD	10 (5 F; 5 M)	16 Sept 2018
BB	6 (2 F; 4 M)	3 Oct 2018
NN	8 (4 F; 4 M)	5 Nov 2018

During the first week after birth, all subjects were habituated to the presence of the observers (E. D. and M. G.) and data collection started around the end of the first week of life. The behavioural patterns of piglets were video-recorded from September to December 2018 using HD/Full HD Sony HDR-XR200 and Panasonic HC-W3580 cameras. We collected and analysed 15.92 h (Mean $\pm$ SE per litter: 5.31 h $\pm$ 0.60) of play-fighting/real-fighting videos for all three litters (Linda's litter 6.50 h, Nina's litter 4.60 h, Black Beauty's litter 4.82 h of videos). During the first week after birth the observers underwent a training period by I.N. and G.C. to become skilled at animal identification and behavioural pattern distinction. Actual data collection lasted from day 6 to day 50 after birth. Behavioural observations took place 6 days per week from 08:00–17:00 h following a rotation that allowed the balance of the observation time slots across litters. During the video recording the observers stayed around 10 m from the piglets and sows. To facilitate the identification of each subject, piglets were marked with animal painting spray (Raider) by using different combinations of colours (i.e., blue, red and green) and symbols (i.e. dots, circles and lines). Marking was renewed every 4–7 days depending on weather conditions.

2.2.2 Video analysis and operational definitions

By using the all-occurrences animal sampling method (Altmann, 1974) we collected 388 play sessions and 108 aggressive interactions between piglets (**Figure 1**). Prior to starting the video analysis, videocoders (M.G. and E.C) were supervised by G.C. and I.N. in behavioural coding and the video analysis started when the interobserver reliability scores measured via Cohen's k reached 0.83. The interobserver reliability between video coders was calculated using the R function 'cohen.cappa' and libraries 'irr' and 'psych' (R version 3.5.3). The videos were analysed frame by frame and slow motion via freeware software VLC 2.2.1 (Jump-to-time extension). For each play fighting session and real fighting session, we extracted from videos the following information: (1) the identity of the subjects involved, (2) individual features (sex, age), (3) behavioural patterns performed (**Table 2**), (4) time of each pattern and session duration (s).

Play fighting



Real fighting



Figure 1. At the top a play fighting session between piglets. The individual on the right performs a pivot and bites (play-bite) another individual (pig B). Pig B performs a play-push and attempts to bite (attempt play bite) Pig A. Pivot is one of the play markers. At the bottom a real fighting session between piglets. The piglet in the center of the group (pig C) repeatedly bites (aggressive bites) another individual (pig D). Pig D flees. There are no play markers in this session.

Table 2. Description of piglets' behavioural patterns recorded in the current study.

Patterns exclusive to play context		
Category	Pattern	Description
Neutral	Flopping	A piglet drops to the ground from the upright position to a sitting or lying position. There is no contact with an object or another individual that could cause the change in position.
	Head tossing	A piglet swings its head from one side to another
	Hopping	A piglet has either its two front feet or all four feet off the ground at one time through a upwards jumping movement. The piglet continues facing the same original direction for the whole of the behaviour
	Leg spreading	A piglet spreads its fore and hind limbs and it moves quickly from side to side
	Nudging	A piglet uses its snout to touch and push another piglet's body (excluding nose-nose contact) without causing the displacement of the contacted piglet
	Playing with object	A piglet manipulates an item or securely holds it in its mouth, shaking it or carrying it around
	Pivoting	A piglet twirls its body on the horizontal plane by a minimum of 90°
	Scampering	A piglet performs two or more forward directed hops in rapid succession
Defensive	Kneeling	A piglet goes down on its forelimb knees
Patterns exclusive to aggressive context		
Category	Pattern	Description
Neutral	Threatening	A piglet arches its back to the opponent or makes a forward movement of its head and stares at the opponent with no physical contact
	Asymmetric parallel	The piglets face the same direction, standing side by side with one of them staying slightly ahead of the other. A piglet ahead moves forward, pushing the opponent away with its shoulder and moves its head away from the opponent
Defensive	Avoiding	A piglet moves away with a depressed tail when the opponent approaches
	Fleeing	A piglet runs away from the opponent
	Withdrawing	A piglet tries to move away from the opponent while the opponent continues to bite it repeatedly
Patterns shared by play and aggressive contexts		
Category	Pattern	Description
Neutral	Head tilting	A piglet turns its head away from the opponent when the opponent passes or approaches
Defensive	Resting	A piglet sits or lies down during the session.
Offensive	Biting	A piglet opens its mouth and closes its teeth on the opponent's flesh

	Head knocking	A piglet hits another individual with its head (while keeping its mouth closed)
	Kicking	A piglet hits with one or both hindlimbs the opponent
	Lifting	A piglet puts its snout or head below the others neck or belly and pushes upwards.
	Mounting	A piglet rises upon the rear of the partner
	Pushing	A piglet presses its head, neck, shoulder or body against the opponent
	Biting attempt	A piglet opens its mouth, directs or turns its head towards the body of the opponent and closes its mouth but misses contact
	Running after	A piglet rapidly follow another individual

Integrated or modified from other ethograms (Newberry and Wood-Gush, 1988, Donaldson et al., 2002, Chaloupková et al., 2007, Rauw, 2013, Horback, 2014, Špinka, 2017, Brown et al., 2018, Cordoni et al., 2021; Collarini et al., 2022).

According to previous reports (Newberry and Wood-Gush, 1988, Rauw, 2013, Horback, 2014, Špinka, 2017, Collarini et al., 2022), we classified as play fighting sessions the interactions where the piglet performed at least one behavioural item that is specifically found in the play context and not in the aggressive context (**Figure 1; Table 2**). Although the relaxed open mouth (play face) is considered a widespread play signal across mammals (Palagi et al., 2016), it is not listed as a play marker in piglets (e.g. Newberry and Wood-Gush, 1988; Rauw, 2013; Horback, 2014; Špinka, 2017). Consistently, we only observed the open mouth associated with attempted bites but we did not observe relaxed open mouth displays (or play faces) during social play. A play session started when a piglet directed any playful pattern (see **Table 2**) towards the littermate and finished when both players stopped the interaction, with one of them moving away or with a third subject interrupting the session. We categorised as real fighting sessions the interactions where there was no play specific behavioural pattern (**Figure 1; Table 2**). A real fight started when a piglet directed any aggressive pattern (see **Table 2**) towards a litter-mate and it ended with one of the opponents moving or fleeing away. When play- or real fighting sessions were interrupted for at least 10 s, we considered the subsequent play- or real-fighting interaction as a new session (Cordoni et al., 2021). For both play- and real fights, the patterns were classified as Offensive (O, unidirectional patterns performed to attack the opponent), Defensive (D, patterns aimed at eluding the contact or attack by another individual) and Neutral (N, neither offensive nor defensive; **Table 2**). For each play fighting and real fighting session, we calculated different indices to evaluate the distribution and variability of the motor patterns performed within the session and the symmetry of the interaction. In particular, we considered the indices listed below.

Poliadic Index: At the individual level, we assessed the number of dyadic and polyadic play- and real-fighting sessions and we determined the Polyadic Index for the two types of session as "the number of polyadic sessions *minus* the number of dyadic

sessions" divided by "the number of polyadic sessions *plus* the number of dyadic sessions". Positive values of Polyadic Index indicate a higher frequency of polyadic over dyadic sessions.

Asymmetry Index (AI): The play Asymmetry Index (Cordoni et al., 2016, Cordoni et al., 2018, Cordoni et al., 2021) was used to quantify the level of play fighting asymmetry. For each interaction we calculated play Asymmetry Index as follows: "the number of offensive patterns by A towards B plus the number of defensive patterns by B towards A" subtracted from "the number of offensive patterns by B towards A plus the number of defensive patterns by A towards B" divided by "the total number of patterns performed by both playmates". The formula of play Asymmetry Index is reported below:

$$pAI = \frac{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) - (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B})}{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) + (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B}) + \text{neutral}_{A+B}}$$

Based on the play Asymmetry Index, we also calculated the aggression Asymmetry Index to quantify the level of real fighting asymmetry. For each interaction we calculated aggression Asymmetry Index as follows: "the number of offensive patterns by A towards B plus the number of defensive patterns by B towards A" subtracted from "the number of offensive patterns by B towards A plus the number of defensive patterns by A towards B" divided by "the total number of patterns performed by both opponents". The formula of aggression Asymmetry Index is reported below:

$$aAI = \frac{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) - (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B})}{(\text{offensive}_{A \rightarrow B} + \text{defensive}_{B \rightarrow A}) + (\text{offensive}_{B \rightarrow A} + \text{defensive}_{A \rightarrow B}) + \text{neutral}_{A+B}}$$

Both play Asymmetry Index and aggression Asymmetry Index range from - 1 to + 1 with main values indicating i) a complete symmetry of the session (zero), ii) a complete asymmetry of the session in favour of A (+1) and, iii) a complete asymmetry of the session in favour of B (-1).

Repetition index: To evaluate the level of repetition of the same pattern within a single real fighting or play fighting session, this index was calculated by dividing the number of different types of patterns that were repeated during a session over the total number of patterns that composed the session, normalized over the session duration.

The indices listed below were adapted from indices used to measure biodiversity in ecological studies (Türkmen and Kazanci, 2010, Morris et al., 2014, Supriatna, 2018). In particular, we considered each play/real- fight session as an 'ecosystem' including individuals (i.e. in our case all the behavioural patterns included

in the session) belonging to different species (i.e. in our case the different types of behavioural patterns included in the session). Based on this approach, we calculated the Shannon and the Pielou indices.

Shannon index (H'): Shannon index is the most common diversity index used in ecological studies and expresses. The richness of the specific ecosystem whilst also taking into account the relative abundance of species (Shannon and Weaver, 1949, Wilsey and Potvin, 2000, Mandaville, 2002, Keylock, 2005, Eliazar and Sokolov, 2012, Daly et al., 2018). The mathematical formula of Shannon index is: s:

$$H' = -\sum[(n_i/N) * (\ln n_i/N)]$$

In particular, n_i is the number of individuals belonging to the species i and N is the total number of individuals in a specific ecosystem. Shannon Index values are generally between zero and five; when they are equal to, or higher than four, it indicates a high level of biodiversity and a balanced ecosystem structure. In our study n_i is represented by numbers of patterns belonging to the type i and N is represented by the total number of patterns composing a session. A high value of Shannon Index indicates a great behavioural pattern variability in terms of different types of patterns performed in a single session.

Pielou Index (J; also known as Species evenness): it derives from the Shannon index and focuses on how individuals are distributed across species (more or less evenly) within a specific ecosystem (Pielou, 1966). The mathematical formula of Pielou index is:

$$J = \frac{H'}{H'_{\max}}$$

H' is the observed value of Shannon index, $H'_{\max}$ is the $\ln S$ with S representing the total number of species. The values of Pielou Index vary between zero and one; when they are close to one it means that individuals are even distributed among species (Pielou, 1966). In the present study S is represented by the total number of different types of behavioural patterns. To calculate the indices and run analyses on them we selected the individuals with at least two sessions of either play- or real fighting, with at least two patterns per session.

2.2.3 Statistics

Owing to normal data distribution (Kolmogorov Smirnov, $n_{\text{individuals}} = 22$, $0.074 \leq p \leq 0.325$), at the individual level we applied the parametric t-test for dependent samples to compare the mean duration and Polyadic Index, play/aggression Asymmetry Index, Shannon Index, Pielou Index, and Repetition Index values between

real- and play fighting. The tests were carried out via SPSS 26.0. The threshold of statistical significance for all tests was set at $\alpha = 0.05$.

2.3 Results

2.3.1 Prediction 1

The mean duration was significantly lower in real- than play fighting sessions (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = 3.949$; $p = 0.001$, 95% CI: 1.249/4.027; **Figure 2**). We detected no significant difference in the Polyadic Index values between real- and play fighting (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = -1.526$; $p = 0.142$, 95% CI: $-0.944/0.145$), which indicates that the proportion of polyadic sessions in real- and play fighting was comparable.

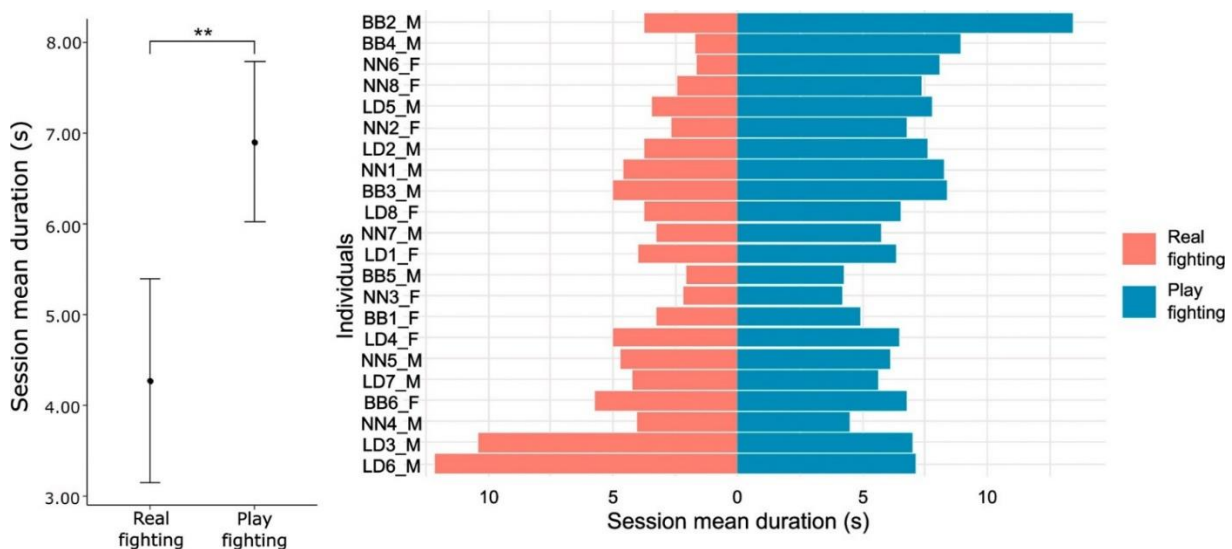


Figure 2. Difference between the mean duration of real fighting and play fighting sessions shown as error bar (left) and bar plot (right). The mean duration was significantly lower in real fighting than play fighting sessions (t-test for dependent samples; $p = 0.001$). Labels on the Y axis indicate the litter code, with piglet number and sex (F=female; M=male); ** = $p < 0.01$.

2.3.2 Prediction 2

The Asymmetry Index was significantly higher in real- than play fighting (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = -13.927$; $p < 0.001$; 95% CI: $-0.723/-0.535$; **Figure 3a**). The level of pattern variability (Shannon Index, H') was significantly lower in real- than in play fighting (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = 4.932$; $p < 0.001$; 95% CI: 0.168/0.414; **Figure 3b**). Individual analysis of pattern evenness (Pielou Index) levels showed no difference between real fighting and play fighting sessions (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = 1.681$; $p = 0.107$; 95% CI: $-0.020/0.191$; **Figure 3c**). Finally, the Repetition Index was significantly higher in real- than in play fighting (t-test for dependent samples, $n_{\text{individuals}} = 22$; $t = -4.102$; $p = 0.001$; 95% CI: $-0.058/0.019$; **Figure 3d**).

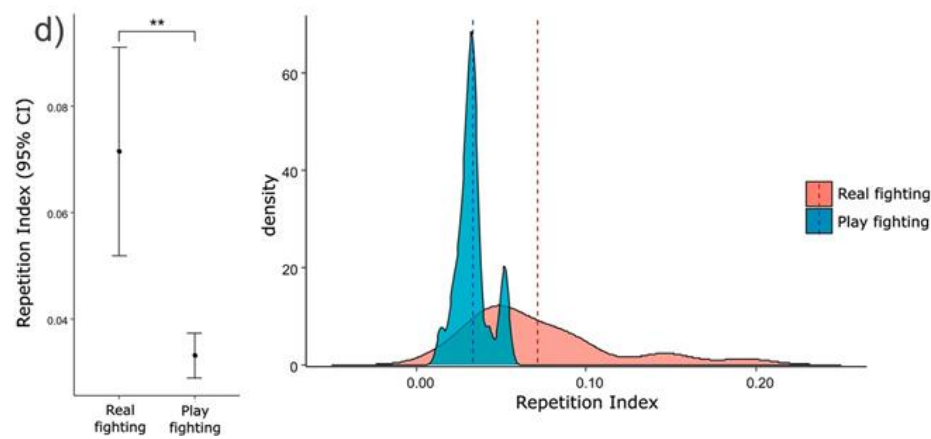
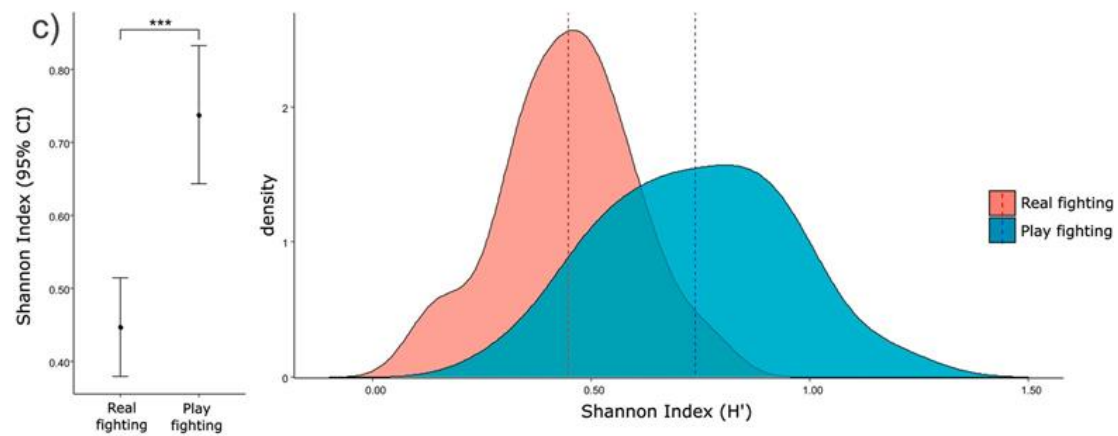
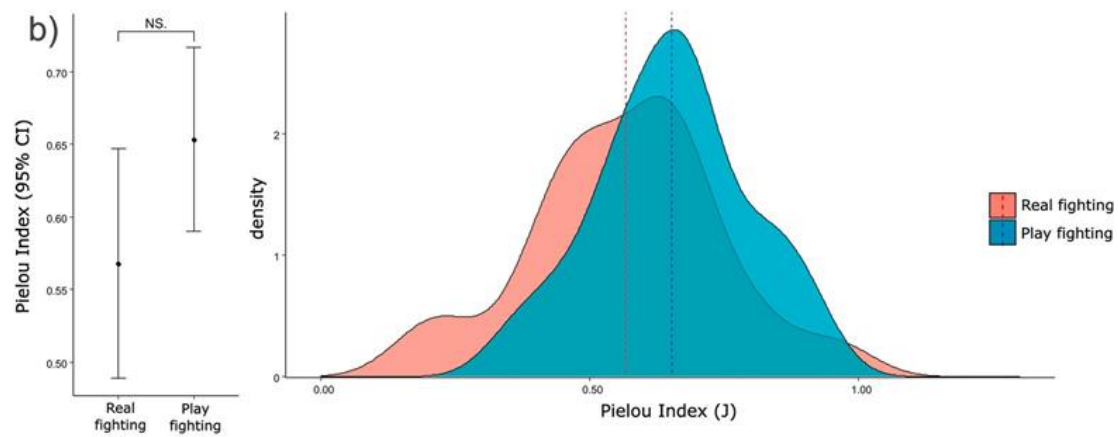
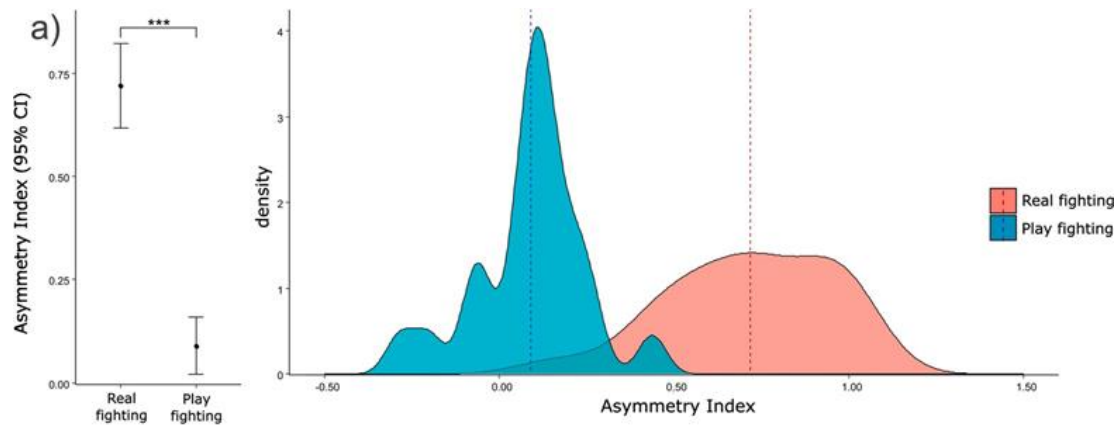


Figure 3. Difference between real fighting and play fighting (verified via t-test for independent samples) in piglets shown as error bar (left) and density plot (right). a) Asymmetry Index values; real fighting shows the highest levels of asymmetry ($p < 0.001$). b) Pielou Index values; real fighting and play fighting show comparable levels of evenness ($p = 0.107$). c) Shannon Index values; real fighting shows the lowest levels of variability ($p < 0.001$). d) Repetition index values: real fighting shows higher levels of repetition ($p = 0.001$). Error bars: 95% confidence interval (bars) around the mean (circles). NS = non significant, $** = p < 0.01$, $*** = p < 0.001$.

2.4 Discussion

In this study, we found that - as expected - play fighting sessions lasted longer than real fighting sessions (*Prediction 1a* supported; **Figure 2**) and there was no difference in the polyadic index values between play fighting and real fighting sessions (*Prediction 1b* supported). We also found that - compared to real fighting - play fighting sessions could be more balanced as informed by the fact that they: i) included more symmetrically exchanged patterns (offensive/defensive; **Figure 3a**) but such patterns were not more uniformly distributed (*Prediction 2a* partly supported; **Figure 3b**). Compared to real fighting, play fighting sessions: i) were more variable in terms of different types of motor patterns within a single session (*Prediction 2b* supported; **Figure 3c**); and ii) showed lower repetition levels (*Prediction 2c* not supported; **Figure 3d**).

The fact that play fighting sessions lasted longer than real fighting sessions (**Figure 2**) might inform the rewarding nature of play. Indeed, according to previous literature, the expression of playful behaviours is supposed to be pleasurable and could be rewarding for individuals involved in such behaviours (Burghardt, 2005, Burghardt, 2011). The pleasure and reward that emerge from playful behaviour could induce the involved individuals to increase the duration of play sessions (Held and Špinka, 2011, van Kerkhof et al., 2013, Pellis et al., 2014). The individuals involved in play fighting sessions could gain more time in the social assessment process by prolonging the duration of these playful interactions (Palagi et al., 2015, Palagi et al., 2019, Bertini et al., 2022).

The lack of a significant difference in the level of 'polyadicity' between play- and real fighting can be explained by the fact that aggressive events in adult pigs can involve more than two individuals, with several pigs (which can be males and/or females) forming gang alliances and carrying out targeted aggression towards other conspecifics (Camerlink et al., 2020). In the piglets under study, we did not observe gang attacks but we observed that more than two individuals (typically three) could be involved in the same aggressive session. The possibility of forming alliances is consistent with the high cognitive abilities of pigs that can include anticipatory and problem-solving skills (Croney and Boysen, 2021, Norscia et al., 2021b), post-conflict management abilities (reconciliation and triadic affiliative contacts after aggression; Cordonì et al., 2022), and the capacity to express, detect and share, others' physiological states (Turner et al., 2001; Jensen, 2002; D'Eath and Turner, 2008; Reimert et al., 2013; Camerlink et al., 2018; Norscia et al., 2021a, Norscia et al., 2021b). Consistently, polyadic conflicts (with alliances) have been observed in

cognitively advanced species, such as wolves and great apes (van Schaik et al., 2004; von Rohr et al., 2012; Cassidy and McIntyre, 2016) in which group mates can form coalitions during agonistic events.

In the piglets under study, play fighting sessions were more symmetrical (higher Asymmetry Index values) than real fighting ones. Increased symmetry levels can enhance the continuation of play, thus increasing the functional benefits that play can have for immature subjects such as motor training, social assessment, and receiving less injuries during contests at a later stage (Byers and Walker, 1995, Pellis and Iwaniuk, 1999, Pellis and Iwaniuk, 2000, Cordoni and Palagi, 2011, Turner et al., 2020). Commonly mentioned strategies that allow the achievement of symmetry are the role reversal (e.g., balancing subordinate/dominance patterns) and self-handicapping (when the most skilled player restrains itself to decrease its advantage over the playmate; Cordoni et al., 2016; Cordoni and Palagi, 2016). However, pigs may use a different strategy. A study by Pellis and Pellis (2016) found that Visayan warty pigs (*Sus cebifrons*) did not show restraint during play, as when one animal gained the advantage they most probably attacked their partner. However, frequently the disadvantaged partners could launch a counter-attack and increase the session balance. Consistently, in the same litters under study, Cordoni et al., 2021 found that only the piglets of similar size could maintain the play session longer, probably because self-restraint was not necessary when neither playmate could outcompete the other by using force. Even though play fighting in piglets can be used as a substitute for real fighting and lead to a similar outcome as real fighting (Cordoni et al., 2021), our results on the Asymmetry Index show that real fighting maintains the highest degree of directionality. Indeed, asymmetrical aggressive events are pivotal to gain a dominant status, as the ranking position of an individual within a group increases as the number of agonistic encounters consistently won by that individual increases (Landau, 1951, Appleby, 1983, Drews, 1993, de Vries, 1995, Kendall, 2021). High ranking individuals have priority in the access to resources (food, mating), which increases their fitness (Clutton-Brock, 1982, Preuschoft and Van Schaik, 2000, Andersen et al., 2004, Sapolsky, 2005, Krebs and Davies, 2012, Norring et al., 2019).

We also found that play fighting possessed higher pattern variability (increased Shannon Index values; **Figure 3c**) than real fighting. Overall, the increased heterogeneity of play fighting patterns is in line with the main characteristics of play, which involve patterns that are variably recombined from other social behaviour domains, including (among others) mating or aggression (Pellis and Pellis, 2017, Pellis et al., 2019, Cordoni et al., 2021). This feature is adaptive to maximize the benefits of play, especially the training for the unexpected (Špinka et al., 2001). As a matter of fact, during play there are no pre-fixed rules so individuals must improvise and quickly counteract in a flexible but appropriate way the actions of the playmate (Pellegrini, 2009, Pellis et al., 2010, Palagi et al., 2016).

The higher repetition levels found in real fighting compared to play fighting may be due to the fact that real fighting - as said above - is more asymmetrical and during aggression individuals can repeat the same offensive pattern (head knocking, pushing,

etc.) over and over to outcompete the opponent. Our result seems not to entirely fit with one of the very criteria that define play (Smith, 1989, Smith, 1997, Burghardt, 2005, Burghardt, 2011). This aspect deserves further investigation in future studies to better understand what other measures can be used to assess the repetition levels and what is the term of comparison.

The comparable levels of evenness (Pielou Index values) in play fighting and real fighting may be the result of the convergence of different features: play fighting sessions were more symmetrical and longer whereas real fighting sessions showed higher levels of repetition of the same patterns. These diverging structural characteristics might have dampened the differences in the uniformity levels between the 'serious' and 'non-serious' context and made evenness a less reliable feature to assess the playful mood in piglets.

In conclusion, this study shows that play fighting sessions in piglets did not fully comply with the features that should allow the differentiation between play and real fighting sessions, for example in terms of repetition levels. This finding is in line with the observation that play fighting in piglets not only resembles but also shares some functions of real fighting such as the establishment of dominance relations (Weller et al., 2020, Cordoni et al., 2021). A similar function has been found in other species, including for example humans and chimpanzees (Smith and Boulton, 1990, Paquette, 1994). Interestingly, in humans it has been hypothesized that play fighting - when polyadic (or coalitional) - can lead to the deployment of coordinated aggression because it is found across a diverse range of hunter-gatherer groups and regularly recruits motor patterns used in lethal raiding (Sugiyama et al., 2018).

Despite the fact that in piglets play fighting can substitute real fighting under certain circumstances (Weller et al., 2020, Cordoni et al., 2021), this study shows that play fighting can still maintain the key features that make play adaptive for the individuals that engage in it. Real fighting increases in piglets whereas social play decreases over the first two months of life, with play fighting also working as a substitute of real fighting (Cordoni et al., 2021, Collarini et al., 2022). Thus, age may in part explain our results showing that play fighting maintains elements of play but also includes elements of real fighting, which will prevail at a later stage. Future studies could focus on the difference between play fighting and real fighting at different ages in the first months of life.

By including more variable and symmetrical patterns, play fighting sessions in a competitive arena can serve any one, or a combination, of the proposed functions of play fighting (motor training, social assessment and training for the unexpected). On a broader perspective, this study can provide the ground for measuring the play structure in a quantitative and repeatable way across species and contexts and to assess whether interactions that are apparently playful can be actually labelled as play.

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2.5 References

- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49(3–4), 227–266. <https://doi.org/10.1163/156853974x00534>
- Andersen, L. B., Hasselstrøm, H., Grønfeldt, V., Hansen, S. E., & Karsten, F. (2004). The relationship between physical fitness and clustered risk, and tracking of clustered risk from adolescence to young adulthood: Eight years follow-up in the Danish Youth and Sport Study. *International Journal of Behavioral Nutrition and Physical Activity*, 1(6). <https://doi.org/10.1186/1479-5868-1-6>
- Appleby, M. C. (1983). The probability of linearity in hierarchies. *Animal Behaviour*, 31(3), 600–608. [https://doi.org/10.1016/s0003-3472\(83\)80084-0](https://doi.org/10.1016/s0003-3472(83)80084-0)
- Bauer, E. B., & Smuts, B. B. (2007). Cooperation and competition during dyadic play in domestic dogs, *Canis familiaris*. *Animal Behaviour*, 73(3), 489–499. <https://doi.org/10.1016/j.anbehav.2006.09.006>
- Bekoff, M. (1975). The communication of play intention: Are play signals functional? *Semiotica*, 15(3), 231–240. <https://doi.org/10.1515/semi.1975.15.3.231>
- Bekoff, M. (2014). The significance of ethological studies: Playing and peeing. In *Domestic Dog Cognition and Behavior* (pp. 59–75). Springer Berlin Heidelberg.
- Bekoff, M., & Allen, C. (1998). Intentional communication and social play: How and why animals negotiate and agree to play. In J. A. Byers (Ed.), *Animal Play: Evolutionary, Comparative, and Ecological Perspectives* (pp. 97–114). Cambridge University Press.
- Bertini, M., Annicchiarico, G., Bresciani, C., Cordonì, G., & Palagi, E. (2022). Playful interactions and facial mimicry in infant bonobos (*Pan paniscus*). *Ethology Ecology & Evolution*, 34(4), 344–359. <https://doi.org/10.1080/03949370.2021.1988723>
- Blackshaw, J. K., Swain, A. J., Blackshaw, A. W., Thomas, F. J. M., & Gillies, K. J. (1997). The development of playful behaviour in piglets from birth to weaning in three farrowing environments. *Applied Animal Behaviour Science*, 55(1–2), 37–49. [https://doi.org/10.1016/s0168-1591\(97\)00034-8](https://doi.org/10.1016/s0168-1591(97)00034-8)
- Blumstein, D. T., Chung, L. K., & Smith, J. E. (2013). Early play may predict later dominance relationships in yellow-bellied marmots (*Marmota flaviventris*). *Proceedings of the Royal Society B: Biological Sciences*, 280(1759), 20130485.
- Brown, S. M., Peters, R., Nevison, I. M., & Lawrence, A. B. (2018). Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. *Applied Animal Behaviour Science*, 198, 36–43. <https://doi.org/10.1016/j.applanim.2017.09.018>
- Burghardt, G. M. (1998). The evolutionary origins of play revisited. In *Animal Play: Evolutionary, Comparative, and Ecological Perspectives* (pp. 3–26).
- Burghardt, G. M. (2005). *The genesis of animal play: Testing the limits*. MIT Press.

Burghardt, G. M. (2010). The comparative reach of play and brain: Perspective, evidence, and implications. *American Journal of Play*, 2, 338–356.

Burghardt, G. M. (2011). In P. Nathan (Ed.), *The Oxford Handbook of the Development of Play*.

Byers, J. A., & Walker, C. (1995). Refining the motor training hypothesis for the evolution of play. *The American Naturalist*, 146, 25–40. <https://doi.org/10.1086/285785>

Camerlink, I., Coulange, E., Farish, M., Baxter, E. M., & Turner, S. P. (2018). Facial expression as a potential measure of both intent and emotion. *Scientific Reports*, 8, 17602. <https://doi.org/10.1038/s41598-018-35905-3>

Camerlink, I., Chou, J.-Y., & Turner, S. P. (2020). Intra-group lethal gang aggression in domestic pigs (*Sus scrofa domestica*). *Animals*, 10(8), 1287. <https://doi.org/10.3390/ani10081287>

Cassidy, K. A., & McIntyre, R. T. (2016). Do gray wolves (*Canis lupus*) support pack mates during aggressive inter-pack interactions? *Animal Cognition*, 19, 939–947. <https://doi.org/10.1007/s10071-016-0994-1>

Chaloupková, H., Illmann, G., Bartoš, L., & Špinka, M. (2007). The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Applied Animal Behaviour Science*, 103, 25–34. <https://doi.org/10.1016/j.applanim.2006.04.020>

Clutton-Brock, T. H. (1982). The functions of antlers. *Behaviour*, 79(1–2), 108–124. <https://doi.org/10.1163/156853982x00201>

Collarini, E., Gioia, M., & Cordoni, G. (2022). Does the domestication syndrome apply to the domestic pig? Not completely. *Animals*, 12(18), 2458. <https://doi.org/10.3390/ani12182458>

Cordoni, G., & Palagi, E. (2011). Ontogenetic trajectories of chimpanzee social play: Similarities with humans. *PLoS ONE*, 6(11), e27344.

Cordoni, G., & Palagi, E. (2016). Aggression and hierarchical steepness inhibit social play in adult wolves. *Behaviour*, 153, 749–766. <https://doi.org/10.1163/1568539x-00003326>

Cordoni, G., Nicotra, V., & Palagi, E. (2016). Unveiling the “secret” of play in dogs (*Canis lupus familiaris*): Asymmetry and signals. *Journal of Comparative Psychology*, 130, 278–287. <https://doi.org/10.1037/com0000035>

Cordoni, G., Norscia, I., Bobbio, M., & Palagi, E. (2018). Differences in play can illuminate differences in affiliation: A comparative study on chimpanzees and gorillas. *PLoS ONE*, 13(3), e0193096. <https://doi.org/10.1371/journal.pone.0193096>

Cordoni, G., Gioia, M., Demuru, E., & Norscia, I. (2021). The dark side of play: Play fighting as a substitute for real fighting in domestic pigs (*Sus scrofa*). *Animal Behaviour*, 175, 21–31. <https://doi.org/10.1016/j.anbehav.2021.02.016>

Cordoni, G., Comin, M., Collarini, E., Robino, C., Chierto, E., & Norscia, I. (2022). Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: Cognitive and functional implications. *Animal Cognition*. <https://doi.org/10.1007/s10071-022-01688-4>

Croney, C. C., & Boysen, S. T. (2021). Acquisition of a joystick-operated video task by pigs (*Sus scrofa*). *Frontiers in Psychology*, 12, 631755. <https://doi.org/10.3389/fpsyg.2021.631755>

D'Eath, R. B., & Turner, S. P. (2008). The natural behaviour of the pig. In *The Welfare of Pigs* (pp. 13–45). Springer.

Daly, A., Baetens, J., & De Baets, B. (2018). Ecological diversity: Measuring the unmeasurable. *Mathematics*, 6(7), 119. <https://doi.org/10.3390/math6070119>

Donaldson, T. M., Newberry, R. C., Špinka, M., & Cloutier, S. (2002). Effects of early play experience on play behaviour of piglets after weaning. *Applied Animal Behaviour Science*, 79(3), 221–231. [https://doi.org/10.1016/s0168-1591\(02\)00138-7](https://doi.org/10.1016/s0168-1591(02)00138-7)

Drews, C. (1993). The concept and definition of dominance in animal behaviour. *Behaviour*, 125(3–4), 283–313. <https://doi.org/10.1163/156853993x00290>

Eliazar, I. I., & Sokolov, I. M. (2012). Measuring statistical evenness: A panoramic overview. *Physica A: Statistical Mechanics and Its Applications*, 391(4), 1323–1353. <https://doi.org/10.1016/j.physa.2011.09.007>

Fagen, R. (1981). *Animal Play Behavior*. Oxford University Press.

Graham, K. L., & Burghardt, G. M. (2010). Current perspectives on the biological study of play: Signs of progress. *The Quarterly Review of Biology*, 85(4), 393–418. <https://doi.org/10.1086/656903>

Held, S. D. E., & Špinka, M. (2011). Animal play and animal welfare. *Animal Behaviour*, 81(5), 891–899. <https://doi.org/10.1016/j.anbehav.2011.01.007>

Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2, 186. <https://doi.org/10.12966/abc.05.08.2014>

Kendall, M. G. (2021). *Rank Correlation Methods* (3rd ed.). Hassell Street Press.

Keylock, C. J. (2005). Simpson diversity and the Shannon-Wiener index as special cases of a generalized entropy. *Oikos*, 109(1), 203–207. <https://doi.org/10.1111/j.0030-1299.2005.13735.x>

Krebs, J. R., & Davies, N. B. (2012). *An Introduction to Behavioural Ecology*. John Wiley & Sons.

Landau, H. G. (1951). On dominance relations and the structure of animal societies: II. Some effects of possible social factors. *The Bulletin of Mathematical Biophysics*, 13(3), 245–262. <https://doi.org/10.1007/bf02477920>

Mandaville, S. M. (2002). *Benthic Macroinvertebrates in Freshwater - Taxa Tolerance Values, Metrics, and Protocols, Project H - 1*. Soil & Water Conservation, Nova Scotia.

Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T., Müller, C., Obermaier, E., Prati, D., Socher, S. A., Sonnemann, I., Wäschke, N., Wubet, T., Wurst, S., & Rillig, M. C. (2014). Choosing and using diversity indices: Insights for ecological applications from the German Biodiversity Exploratories. *Ecology and Evolution*, 4(18), 3514–3524. <https://doi.org/10.1002/ece3.1155>

Newberry, R.C., & Wood-Gush, D.G.M. (1988). Development of some behaviour patterns in piglets under semi-natural conditions. *Animal Science*, 46(1), 103–109. <https://doi.org/10.1017/s0003356100003160>

Norring, M., Valros, A., Bergman, P., Marchant-Forde, J.N., & Heinonen, M. (2019). Body condition, live weight and success in agonistic encounters in mixed parity groups of sows during gestation. *Animal*, 13(2), 392–398. <https://doi.org/10.1017/s1751731118001453>

Norscia, I., & Palagi, E. (2011). When play is a family business: adult play, hierarchy, and possible stress reduction in common marmosets. *Primates*, 52(2), 101–104. <https://doi.org/10.1007/s10329-010-0228-0>

Norscia, I., & Palagi, E. (2015). The socio-matrix reloaded: From hierarchy to dominance profile in wild lemurs. *PeerJ*, 3, e729. <https://doi.org/10.7717/peerj.729>

Norscia, I., Coco, E., Robino, C., Chierito, E., & Cordoni, G. (2021a). Yawn contagion in domestic pigs (*Sus scrofa*). *Scientific Reports*, 11, 1851. <https://doi.org/10.1038/s41598-020-80545-1>

Norscia, I., Collarini, E., & Cordoni, G. (2021b). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>

Palagi, E. (2011). Playing at every age: Modalities and potential functions in non-human primates.

Palagi, E., & Cordoni, G. (2012). The right time to happen: Play developmental divergence in the two *Pan* species. *PLoS One*, 7(12), e52767. <https://doi.org/10.1371/journal.pone.0052767>

Palagi, E., Antonacci, D., & Cordoni, G. (2007). Fine-tuning of social play in juvenile lowland gorillas (*Gorilla gorilla gorilla*). *Developmental Psychobiology*, 49(5), 433–445. <https://doi.org/10.1002/dev.20219>

Palagi, E., Norscia, I., & Spada, G. (2014). Relaxed open mouth as a playful signal in wild ring-tailed lemurs: Relaxed open mouth as a play signal in lemurs. *American Journal of Primatology*, 76(11), 1074–1083. <https://doi.org/10.1002/ajp.22294>

Palagi, E., Nicotra, V., & Cordoni, G. (2015). Rapid mimicry and emotional contagion in domestic dogs. *Royal Society Open Science*, 2(8), 150505. <https://doi.org/10.1098/rsos.150505>

Palagi, E., Burghardt, G.M., Smuts, B., Cordoni, G., Dall'Olio, S., Fouts, H.N., R̃eha'kova'-Petr̃, M., Sivi, S.M., & Pellis, S.M. (2016). Rough-and-tumble play as a window on animal communication: Social play and communication in animals. *Biological Reviews*, 91(2), 311–327. <https://doi.org/10.1111/brv.12172>

Palagi, E., Norscia, I., Pressi, S., & Cordoni, G. (2019). Facial mimicry and play: A comparative study in chimpanzees and gorillas. *Emotion*, 19(4), 665–681. <https://doi.org/10.1037/emo0000476>

Paquette, D. (1994). Fighting and playfighting in captive adolescent chimpanzees. *Aggressive Behavior*, 20(1), 49–65. [https://doi.org/10.1002/1098-2337\(1994\)20:1<49::AID-AB2480200107>3.0.CO;2-C](https://doi.org/10.1002/1098-2337(1994)20:1<49::AID-AB2480200107>3.0.CO;2-C)

Pellegrini, A.D. (2009). *The role of play in human development*. Oxford University Press.

Pellis, S.M., & Iwaniuk, A.N. (1999). The problem of adult play fighting: A comparative analysis of play and courtship in primates. *Ethology*, 105(9), 783–806. <https://doi.org/10.1046/j.1439-0310.1999.00457.x>

Pellis, S.M., & Iwaniuk, A.N. (2000). Adult-adult play in primates: Comparative analyses of its origin, distribution and evolution. *Ethology*, 106(12), 1083–1104. <https://doi.org/10.1046/j.1439-0310.2000.00627.x>

Pellis, S.M., & Pellis, V.C. (1987). Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat (*Rattus norvegicus*). *Aggressive Behavior*, 13(4), 227–242. [https://doi.org/10.1002/1098-2337\(1987\)13:4<227::AID-AB2480130406>3.0.CO;2-C](https://doi.org/10.1002/1098-2337(1987)13:4<227::AID-AB2480130406>3.0.CO;2-C)

Pellis, S.M., & Pellis, V.C. (1996). On knowing it's only play: The role of play signals in play fighting. *Aggression and Violent Behavior*, 1(3), 249–268. [https://doi.org/10.1016/1359-1789\(95\)00016-x](https://doi.org/10.1016/1359-1789(95)00016-x)

Pellis, S.M., & Pellis, V.C. (2009). *The playful brain: Venturing to the limits of neuroscience*. Oneworld.

Pellis, S.M., & Pellis, V.C. (2016). Play fighting in Visayan warty pigs (*Sus cebifrons*): Insights on restraint and reciprocity in the maintenance of play. *Behaviour*, 153(6-7), 727–747.

Pellis, S.M., & Pellis, V.C. (2017). What is play fighting and what is it good for? *Learning & Behavior*, 45(4), 355–366. <https://doi.org/10.3758/s13420-017-0264-3>

Pellis, S.M., Pellis, V.C., & Bell, H.C. (2010). The function of play in the development of the social brain. *American Journal of Play*, 2(3), 278–296.

Pellis, S.M., Pellis, V.C., & Himmler, B.T. (2014). How play makes for a more adaptable brain: A comparative and neural perspective. *American Journal of Play*, 7(1), 73–98.

Pellis, S.M., Pellis, V.C., Pelletier, A., & Leca, J.-B. (2019). Is play a behavior system, and, if so, what kind? *Behavioural Processes*, 160, 1–9. <https://doi.org/10.1016/j.beproc.2018.12.011>

Petersen, H.V., Vestergaard, K., & Jensen, P. (1989). Integration of piglets into social groups of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 23(3), 223–236. [https://doi.org/10.1016/0168-1591\(89\)90113-5](https://doi.org/10.1016/0168-1591(89)90113-5)

Petrů, M., Špinka, M., Charvátová, V., & Lhota, S. (2009). Revisiting play elements and self-handicapping in play: A comparative ethogram of five Old World monkey species. *Journal of Comparative Psychology*, 123(3), 250–263. <https://doi.org/10.1037/a0016217>

Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13(2), 131–144. [https://doi.org/10.1016/0022-5193\(66\)90013-0](https://doi.org/10.1016/0022-5193(66)90013-0)

Preuschoft, S., & Van Schaik, C.P. (2000). Dominance and communication. In W.G. Axelrod (Ed.), *Natural Conflict Resolution* (pp. 77–105).

Rauw, W.M. (2013). A note on the consistency of a behavioral play marker in piglets. *Journal of Animal Science and Biotechnology*, 4(1), 33. <https://doi.org/10.1186/2049-1891-4-33>

Rohr, V., Koski, C. R., Burkart, S. E., Caws, J. M., Fraser, C., Ziltener, O. N., & Van Schaik, A. (2012). Impartial third-party interventions in captive chimpanzees: A reflection of community concern. *PLoS One*, 7. <https://doi.org/10.1371/journal.pone.0039011>

Ruis, M. A. W., te Brake, J. H. A., van de Burgwal, J. A., de Jong, I. C., Blokhuis, H. J., & Koolhaas, J. M. (2000). Personalities in female domesticated pigs: Behavioural and physiological indications. *Applied Animal Behaviour Science*, 66(1), 31–47. [https://doi.org/10.1016/S0168-1591\(99\)00070-2](https://doi.org/10.1016/S0168-1591(99)00070-2)

Rushen, J., & Pajor, E. (1987). Offence and defence in fights between young pigs (*Sus scrofa*). *Aggressive Behavior*, 13(6), 329–346. [https://doi.org/10.1002/1098-2337\(1987\)13:6<329::AID-AB2480130602>3.0.CO;2-3](https://doi.org/10.1002/1098-2337(1987)13:6<329::AID-AB2480130602>3.0.CO;2-3)

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science*, 308(5722), 648–652. <https://doi.org/10.1126/science.1106477>

Schmitt, O., Baxter, E. M., Boyle, L. A., & O'Driscoll, K. (2019). Nurse sow strategies in the domestic pig: II. Consequences for piglet growth, suckling behaviour and sow nursing behaviour. *Animal*, 13(3), 590–599. <https://doi.org/10.1017/S1751731118001702>

Shannon, C. E., & Weaver, W. (1949). *The Mathematical Theory of Communication*. University of Illinois Press.

Šilerová, J., Špinka, M., Šárová, R., & Algers, B. (2010). Playing and fighting by piglets around weaning on farms employing individual or group housing of lactating sows. *Applied Animal Behaviour Science*, 124(1-2), 83–89. <https://doi.org/10.1016/j.applanim.2010.02.003>

Smith, P. K. (1989). The role of rough-and-tumble play in the development of social competence: Theoretical perspectives and empirical evidence. In T. Berndt & G. Ladd (Eds.), *Social Competence in Developmental Perspective* (pp. 239–255). Springer.

Smith, P. K. (1997). Play fighting and real fighting: Perspectives on their relationship. In A. Sluckin (Ed.), *Play and Aggression: A Study of Rattling and Clashing in Children* (pp. 49–62).

Smith, P. K., & Boulton, M. (1990). Rough-and-tumble play, aggression and dominance: Perception and behaviour in children's encounters. *Human Development*, 33(5), 271–282. <https://doi.org/10.1159/000276524>

Špinka, M. (2017). Behaviour of pigs. In P. Jensen (Ed.), *The Ethology of Domestic Animals: An Introductory Text* (3rd ed., pp. 214–227). CAB International.

Špinka, M., Newberry, R. C., & Bekoff, M. (2001). Mammalian play: Training for the unexpected. *The Quarterly Review of Biology*, 76(2), 141–168. <https://doi.org/10.1086/393866>

Sugiyama, M. S., Mendoza, M., White, F., & Sugiyama, L. (2018). Coalitional play fighting and the evolution of coalitional intergroup aggression. *Human Nature*, 29(3), 219–244. <https://doi.org/10.1007/s12110-018-9319-1>

Supriatna, J. (2018). Biodiversity indexes: Value and evaluation purposes. *E3S Web of Conferences*, 48, 01001. <https://doi.org/10.1051/e3sconf/20184801001>

Takahashi, L. K., & Lore, R. K. (1983). Play fighting and the development of agonistic behavior in male and female rats. *Aggressive Behavior*, 9(3), 217–227. [https://doi.org/10.1002/1098-2337\(1983\)9:3<217::AID-AB2480090303>3.0.CO;2-4](https://doi.org/10.1002/1098-2337(1983)9:3<217::AID-AB2480090303>3.0.CO;2-4)

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: Pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences*, 31(10), 463–469. <https://doi.org/10.1016/j.tips.2010.06.008>

Türkmen, G., & Kazancı, N. (2010). Applications of various biodiversity indices to benthic macroinvertebrate assemblages in streams of a national park in Turkey. *Review of Hydrobiology*.

Turner, S. P., Horgan, G. W., & Edwards, S. A. (2001). Effect of social group size on aggressive behaviour between unacquainted domestic pigs. *Applied Animal Behaviour Science*, 74(3), 203–215. [https://doi.org/10.1016/S0168-1591\(01\)00168-X](https://doi.org/10.1016/S0168-1591(01)00168-X)

Turner, S. P., Weller, J. E., Camerlink, I., Arnott, G., Choi, T., Doeschl-Wilson, A., & Farish, M. (2020). Play fighting social networks do not predict injuries from later aggression. *Scientific Reports*, 10(1), 15486. <https://doi.org/10.1038/s41598-020-72477-7>

van Kerkhof, L. W. M., Damsteegt, R., Trezza, V., Voorn, P., & Vanderschuren, L. J. M. J. (2013). Social play behavior in adolescent rats is mediated by functional activity in medial prefrontal cortex and striatum. *Neuropsychopharmacology*, 38(10), 1899–1909. <https://doi.org/10.1038/npp.2013.83>

van Schaik, C. P., Pandit, S. A., & Vogel, E. R. (2004). A model for within-group coalitionary aggression among males. *Behavioral Ecology and Sociobiology*, 57(2), 101–109. <https://doi.org/10.1007/s00265-004-0818-1>

Vanderschuren, L. J. (2010). How the brain makes play fun. *American Journal of Play*, 2(3), 315–337.

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience and Biobehavioral Reviews*, 70, 86–105. <https://doi.org/10.1016/j.neubiorev.2016.07.025>

de Vries, H. (1995). An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Animal Behaviour*, 50(6), 1375–1389. [https://doi.org/10.1016/0003-3472\(95\)80053-0](https://doi.org/10.1016/0003-3472(95)80053-0)

Weller, J. E., Turner, S. P., Farish, M., Camerlink, I., & Arnott, G. (2020). The association between play fighting and information gathering during subsequent contests. *Scientific Reports*, 10(1), 1133. <https://doi.org/10.1038/s41598-020-58063-x>

Wilsey, B. J., & Potvin, C. (2000). Biodiversity and ecosystem functioning: Importance of species evenness in an old field. *Ecology*, 81(4), 887. <https://doi.org/10.2307/177163>

Worsaae, H., & Schmidt, M. (1980). Plasma cortisol and behaviour in early weaned piglets. *Acta Veterinaria Scandinavica*, 21(4), 640–657. <https://doi.org/10.1186/BF03546852>

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3. Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: cognitive and functional implications

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Abstract

In social mammals, conflict resolution involves the reunion of former opponents (aggressor and victim) after an aggressive event (reconciliation) or post-conflict triadic contacts with a third party, started by either opponent (solicited-TSC) or spontaneously offered by the third party (unsolicited-TUC). These post-conflict strategies can serve different functions, including consolation (specifically when TUCs reduce the victim's anxiety). We investigated the possible presence and modulating factors of such strategies on semi-free ranging pigs (*Sus scrofa*; $N = 104$), housed at the ethical farm *Parva Domus* (Cavagnolo, Italy). Kinship was known. Reconciliation was present and mainly occurred between weakly related pigs to possibly improve tolerant cohabitation. Triadic contacts (all present except aggressor TSCs) mostly occurred between close kin. TSCs enacted by victims reduced neither their post-conflict anxiety behaviors nor further attacks by the previous aggressor, possibly because TSCs remained largely unreciprocated. TUCs towards aggressors did not reduce aggressor post-conflict anxiety but limited aggression redirection towards third parties. TUCs towards the victim reduced the victim but not the third-party's anxiety. However, TUCs may also provide inclusive fitness benefits to third parties by benefiting close kin. In sum, pigs engaged in non-random solicited/unsolicited triadic contacts, which suggests that pigs might possess socio-emotional regulation abilities to change their own or others' experience and elements of social appraisal, necessary to detect the emotional arousal of relevant others and (in case of TUCs) take the agency to restore homeostasis.

3.1 Introduction

The *Social Intelligence Hypothesis* posits that social cognition in group-living vertebrates develops in response to the structural complexity of the group and, consequently, to the challenges that the individuals face for social living (Byrne and Whiten 1988; Dunbar and Shultz 2007). According to the relational model proposed by de Waal (2000) one of the main challenges of social animals is to manage the conflicts over resources that inevitably arise within groups and that - if not resolved - may lead to group disruption; thus, an aggressive event can have cascading consequences that affect all group members (Schino and Sciarretta 2015; Pallante et al. 2016).

Several species have developed post-conflict behavioral strategies to preserve group integrity (see **Figure 1**), namely: (i) *reconciliation*, which is defined as the first affiliative contact exchanged between the two opponents right after the end of the aggression (de Waal and van Roosmaleen 1979); and (ii) *triadic affiliation*, which is defined as the first affiliative contact exchanged between one (or both) of the opponents and an uninvolved third party (Romero et al. 2009, 2011). In particular, triadic affiliations can be divided into two types: (i) '*solicited*' if initiated by the victim or the aggressor and directed towards a third party (de Waal and Aureli 1996; de Waal 2000; Palagi and Cordoni 2009); and (ii) '*unsolicited*' if spontaneously initiated by a third party and directed towards the victim or the aggressor (de Waal and Preston 2017). These two types of triadic affiliation can underlie different functions and cognitive abilities (Fraser et al. 2009; Romero et al. 2009, 2011; Cordoni and Palagi 2015; de Waal and Preston 2017).

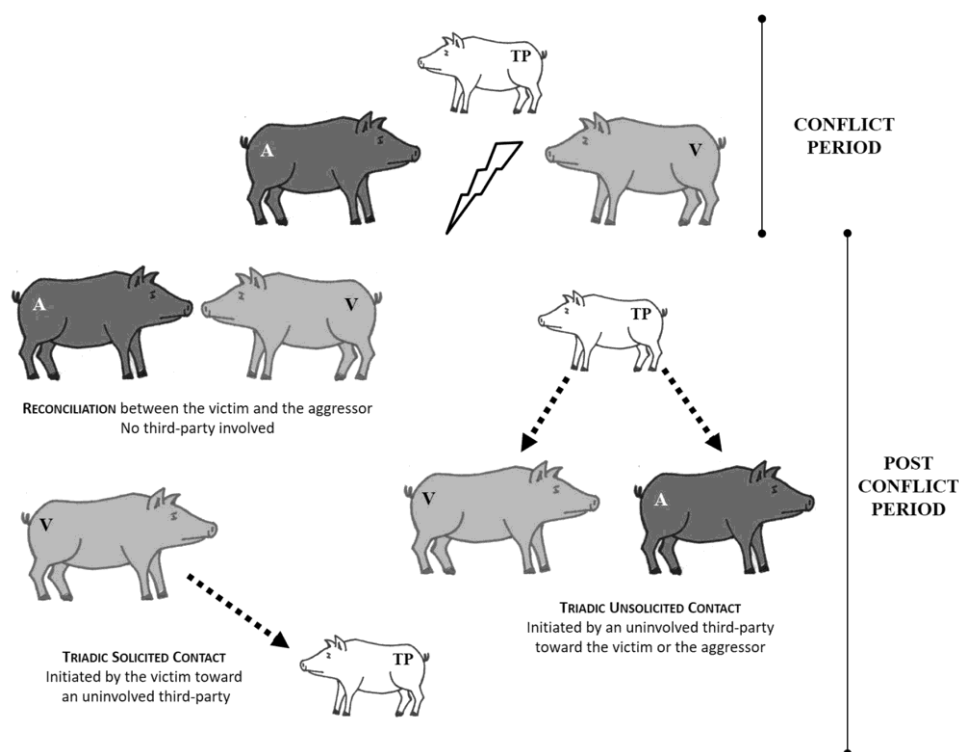


Figure 1. Graphical summary of post-conflict mechanisms observed in the study pig. Legend: A Aggressor, V Victim; TP Third-Party.

Here below we define and detail the above-mentioned post-conflict strategies. Because no direct linkage between psychological processes and behavioral manifestations can be unambiguously delineated (Leavens et al. 2019), we reported the main hypotheses on the cognitive mechanisms that might be (although not exclusively) implicated in post-conflict behavioral strategies.

3.1.1 Reconciliation

Reconciliation is technically defined as the first affiliative contact exchanged between the former opponents (i.e. aggressor and aggression's recipient or victim) within a few minutes (generally 2-min) after a conflict (de Waal and van Roosmaleen 1979; Cordoni et al. 2006; Cordoni and Palagi 2008; Cordoni and Norscia 2014). This phenomenon has been described in various social species, including birds (e.g. parrots, Ikkatai et al. 2016; crows, Sima et al. 2018), a marsupial species (red-necked wallabies, Cordoni and Norscia 2014) and different placental mammals, particularly human and non-human primates (for review see Norscia and Palagi 2016). Reconciliation is a dyadic interaction between two social agents (the former opponents) that might require elements of second-person (or hereafter, 'second-subject') participatory capabilities (in the broader sense; *sensu* de Jaegher et al. 2010). From a functional point of view - among other effects (Aureli 1997; Romero et al. 2009; McFarland and Majolo 2011) - reconciliation can work in repairing the social relationship between the opponents potentially damaged by the aggression, especially with valuable partners such as kin or friends (*Valuable Relationship Hypothesis*, de Waal and Aureli 1997; Wittig and Boesch 2005). From a cognitive point of view, it has been hypothesized that reconciliation might be based on individual recognition and implicit memory of previously encountered subjects (Cords and Thurnheer 1993; Aureli et al. 2002). These abilities might allow animals to behave appropriately and establish or maintain preferential social bonds with specific companions (Gheusi et al. 1994; Massen 2017; Yorzinski 2017). Hence, reconciliation may be not randomly distributed among dyads of opponents and be skewed by the social bond between aggressor and victim (de Waal and Aureli 1997). This might require the further ability to attribute different social values to others (Swallow and Kuiper 1988; Taylor et al. 1995).

3.1.2 Triadic affiliation

Triadic solicited contacts occur when one of the former opponents (victim or aggressor) approaches an uninvolved third party and starts an affiliative contact with her/him (de Waal and Aureli 1996; de Waal 2000; Palagi and Cordoni 2009). From a functional point of view, solicited contacts can indeed regulate the former opponent's experience because they can (i) decrease the probability to receive further aggression from other group members (*Victim Protection Hypothesis*, Palagi and Norscia 2013; Palagi et al. 2014) and/or (ii) reduce self-anxiety (especially if victims; *Self Anxiety*

Reduction Hypothesis; Palagi and Cordoni 2009; McFarland and Majolo 2012; Puga-Gonzalez et al. 2014).

From a social cognition point of view, it can be hypothesized that solicited contacts - although involving a third party - may be still considered dyadic relations (*sensu* Freiwald 2020). To occur, solicited contacts may not necessarily require that a non-participant subject witnesses the interaction between two other social agents (conflict opponents). In this respect, solicited contacts might be a form of *intrinsic* social regulation (*sensu* Zaki and Williams 2013), as an individual (the former opponent) initiates a social contact to possibly regulate *its own* experience. Because the response of the contacted subject can make a difference in such regulation (Zaki and Williams 2013), the contacted third party may not be a random subject (but for example a socially close one; Cordoni et al. 2006; Palagi et al. 2008).

Triadic unsolicited contacts occur when an uninvolved third party spontaneously approaches and starts an affiliative contact with one of the former opponents (de Waal and Preston 2017). The functional effect produced by unsolicited triadic contacts can depend on which one of the former opponents (aggressor or victim) is approached (Romero et al. 2009, 2011; Cordoni and Palagi 2015; Pérez-Manrique and Gomila 2018). When unsolicited triadic contacts are offered to the former aggressor they mostly reduce the risk of further attacks (*Appeasement Hypothesis*; Das 2000; Romero et al. 2011; Cordoni and Palagi 2015). When triadic contacts are engaged with the victim, they may protect the victim against renewed aggression (*Victim Protection Hypothesis*) and reduce its anxiety (*Consolation Hypothesis*; de Waal and van Roosmaleen 1979; Fraser et al. 2008; Fraser and Bugnyar 2010; Romero and de Waal 2010; Palagi and Norscia 2013). Specifically, unsolicited triadic affiliation directed to the victim has defined 'consolation' if and only if it induces a decrease in anxiety levels in the victim in the few minutes following the affiliative interaction (Fraser et al., 2009; de Waal and Preston 2017).

From a social cognition point of view, it may be hypothesized that unsolicited contact might imply a further cognitive step because the interaction between two social agents (the opponents while fighting) is detected and processed by a non-participant subject (uninvolved third party). Recent neuroscientific investigation has found that in primates, for example, direct social interactions and observed social interactions are processed - at least in part - by different brain cortical areas (Freiwald 2020). Furthermore, different regulatory mechanisms may be present (*as per* Zaki and Williams 2013) because it is the bystander that takes agency and actively approaches either one of the former opponents, to possibly change its own experience (intrinsic regulation) and/or the experience of the contacted subject (extrinsic regulation).

When the spontaneous triadic contact by third-party works in reducing the emotional arousal in the contacted individual further cognitive mechanisms may be hypothesized, including possible intrinsic motivation and prosocial function (de Waal and Preston 2017). The process can require elements of social appraisal, through

which an individual's appreciation of a social partner's emotional behavior toward a shared referent regulates the individual's subsequent behavior in relation to such referent (Walle et al. 2017), in this case the aggressive event. Perceiving others' arousal can indeed generate arousal in the observer and induce other-oriented behavior via emotional resonance (Decety et al. 2016). Both the Mirror Neuron System and the Perception-Action Mechanism (PAM) foresee that shared representations of actions may lead to shared representations of the emotions underlying such actions, with the process being modulated by the action goal (MNS: Schütz-Bosbach and Prinz 2015; Caruana 2019) and the observer's experience (PAM: de Waal and Preston 2017). As a first consequence, the resulting behavior of the observer can be implicitly aimed at reducing the divergence between the observer's actual internal state and the observer's prediction of the other subject's emotional state (Prochazkova and Kret 2017). A further consequence is that unsolicited post-conflict contacts may be influenced by individual experience and occur more frequently between closely bonded subjects compared to other dyads (Palagi et al. 2020).

3.2 Focus of the study and predictions

In this study, we provide a comprehensive investigation of the post-conflict mechanisms possibly present in semi-free ranging domestic pigs (*Sus scrofa*), a cognitively advanced species that is able to discriminate familiar individuals and objects, shows sensitivity to the internal states of others and proactively responds to others' distress (Reimert et al. 2013, 2015; Marino and Colvin 2015; Goumon and Špinka 2016; Camerlink et al. 2018; Norscia et al. 2021a, 2021b). Domestic pig reared under semi-natural conditions can perform social behavioral patterns that are typical of its wild counterpart (i.e. wild boar; Jensen, 1986; Stolba and Wood-Gush, 1989). A large array of social interactions between pigs rely on olfaction: nose-to-body and nose-to-nose contacts serve a social exploration and recognition function in affiliative contexts (Camerlink and Turner, 2013; Camerlink et al. 2014). Vocal and body postures are used for inter-individual interactions and communication as well (d'Eath and Turner 2009; Horback 2014). In extensive farms, sows - usually kin related - can form small sub-groups (sounders) including their offspring. Adult females can synchronize their foraging and resting activities and can cooperate in piglet defense (Stolba and Wood-Gush 1989; d'Eath and Turner 2009). Sows leave only temporarily their sounder at the end of pregnancy (about 115 days) to search for a suitable farrowing place and build the nest (Jensen 1986). Mature males can stay in proximity of their conspecifics especially during the mating period but young adults can form stable bachelor groups (Jensen 1982, 2002; Stolba and Wood-Gush 1989; d'Eath and Turner 2009; Dalmau et al. 2020). The different ranking positions are quickly defined through aggression that is generally won by heavier individuals (D'Eath 2002; Andersen et al. 2004; Norring et al. 2019). In well-established groups, subordinates try to limit aggression by avoiding dominant individuals (Jensen 1982). Nevertheless,

aggressive behavior can occur and cause an increase in anxiety (Norscia et al. 2021a, b), physiological stress (Arey and Edwards 1998) and social uncertainty (Cords and Aureli 2000) in group members.

Here, we resume the functionalist framework common to animal post-conflict studies, but we also provide possible insights on whether the presence and modulation of different post-conflict behaviors might support the proposed hypotheses on the cognitive abilities possibly underlying such behaviors. Hence, we tested some hypotheses on post-conflict management in this species and we formulated the following predictions.

3.2.1 Prediction 1: Reconciliation

Pigs are able to distinguish familiar from unfamiliar subjects in large groups and they can also recognize group fellows after several weeks of separation (Kristensen et al. 2001; Turner et al. 2001; McLeman et al. 2005). Thus, domestic pigs may be capable of individual recognition and implicit memory to engage with specific group mates in post-conflict reunions. If so, reconciliation should occur in *Sus scrofa* (*Prediction 1a*). In semi-free ranging and feral pigs, the main social unit is usually formed by kin-related individuals (i.e. generally, two or four related sows with their most recent litters and juvenile subjects of previous litters) that establish preferential social bonds (Jensen 1982, 2002; Graves 1984; Stolba and Wood-Gush 1984; D'Eath and Turner 2009). Hence, pigs may be able to attribute different social values to others (Goumon et al. 2020). If so, the reconciliation should not be randomly distributed across dyads of opponents. In this view, if reconciliation is more frequent when the conflict occurs between closely related than unrelated or less closely related pigs, we can support the *Valuable Relationship Hypothesis* (*Prediction 1b*).

3.2.2 Prediction 2: Triadic solicited contacts

The presence or proximity of other conspecifics has an effect on how pigs cope with stressful situations (Reimert et al. 2014). Thus, pigs might have elements of implicit regulation (sensu Zaki and Williams 2013). If so, we predict to find solicited triadic contacts in pigs (*Prediction 2a*). Since (i) the effectiveness of social regulation also depends on which subject is contacted (Zaki and Williams 2013) and (ii) pigs can establish preferential social bonds with certain fellows (Jensen 1982, 2002; Graves 1984; Stolba and Wood-Gush 1984; D'Eath and Turner 2009), we predicted that solicited triadic contacts occur most frequently between closely-related pigs (*Prediction 2b*). In semi-free-ranging pigs post-conflict affiliation also involves bystanders and social contacts can reduce individual anxiety levels measured via self-directed behaviors (Norscia et al. 2021b). Based on these evidences, if solicited affiliation reduces the anxiety in the opponents, we can support the *Self Anxiety Reduction Hypothesis* (*Prediction 2c*). Moreover, if solicited contacts - via this calming effect - reduce the levels of renewed aggression in the victim, we can support the *Victim Protection Hypothesis* (*Prediction 2d*).

3.2.3 Prediction 3: Triadic unsolicited contacts

Pigs show abilities of individual and object discrimination and understanding of others' cues (Marino and Colvin 2015; Camerlink et al. 2018). Moreover, pigs are sensitive to the physiological and emotional state of others (Norscia et al. 2021a), can respond to others' distress via making contact or moving in proximity (Reimert et al. 2013; Goumon and Špinka 2016; Norscia et al. 2021b) and show emotional contagion especially in negative situations (Reimert et al. 2015). Hence, pigs might have regulatory mechanisms and elements of social appraisal (*sensu* Zaki and Williams 2013). Moreover, in pigs post-conflict arousal experienced by both former opponents and bystanders is buffered by affiliation, which restores baseline levels within three minutes (Norscia et al. 2021b). Hence, we expected that pigs would show unsolicited triadic contacts (*Prediction 3a*). Moreover, we expected that pigs would especially engage in unsolicited triadic contact with closely-related individuals to make social regulation more effective (*Prediction 3b*). Finally, if unsolicited triadic contacts directed to the aggressor reduce the probability of renewed attacks on other group members or on the victim, we can support the *Appeasement Hypothesis* (*Prediction 3c*) and the *Victim Protection Hypothesis* (*Prediction 3d*), respectively. Moreover, if unsolicited affiliation reduces the anxiety levels in the victim, we can support the *Consolation Hypothesis* (*Prediction 3e*).

3.3 Methods

3.3.1 The study group

The study was carried out on a group of semi-free ranging domestic pigs composed of 104 adult individuals (54 males and 50 females, 7–22 months of age) belonging to three different breeds: Parma Black, Large White and Piedmont Black. The pigs were housed at the ethical farm “*Parva Domus*” (Cavagnolo, Turin - Italy) in a woodland natural area of about 13 ha. Ethical farms make important efforts to enhance animal welfare by rearing animals in a natural or semi-natural environment where they are able to (i) freely move and behave according to their specific behavioral repertoire, (ii) integrate their diet with the natural food and (iii) follow their natural day/night cycle. The pigs under study received food pellets (Ciclo Unico P, SILDAMIN®) each morning between 8:30–10:30 am but they also freely foraged throughout the area. The water was available *ad libitum*. The males living in the group were castrated during their first three days of life, while the reproductive male was separated from the rest of the group. Due to the summer culling suspension (June/September) and the subsequent low culling rates (usually one individual per week), all but eight pigs were available for the whole data collection period.

3.3.2 Kinship determination and genetical analyses

Owing to controlled reproduction, kinship (when present) varied from second cousins to full siblings. The different breeds, sizes, and marks allowed the reliable identification of the different generations. However, different mothers and fathers could be related (e.g. siblings or cousins). Therefore, to distinguish between distantly related animals from more closely related ones, genetic analyses were carried out on 31 pigs (2–3 individuals sampled from different sibling generations) at the forensic genetic lab of the Department of Public Health Sciences and Pediatrics (University of Torino). Pig's hair samples were collected by the farmer during the usual weekly visit of a veterinarian. Hair samples were grasped using small tweezers (similar to used in human eyebrows). DNA was extracted by hair bulbs (collected during the study period) via QIAmp DNA Investigator Kit (Qiagen; www.qiagen.com) following the provider's protocol. 11 autosomic STRs were amplified via multiplex PCR Animal Type Pig PCR amplification kit (<http://www.biotype.de>; Biotype AG, Dresden, Germany). Genetic profile typing was obtained via capillary electrophoresis with SeqStudio system (Thermo Fisher Scientific; www.thermofisher.com). Allele frequencies and kinship index (0.08) were set on the basis of a mixed sample of domestic pigs ($n = 412$), consisting of commercial lines commonly used in the production process (Caratti et al. 2010). The mutation rate for all markers was set at 0.002. For each possible dyad of pigs an unspecific kinship search was performed using Familias 3.1.5 "Blind Search" Module (Kling et al. 2014). Likelihood ratio (LR) was calculated for sibling, half-sibling, 1st cousin, and 2nd cousin relationships, scaled versus unrelated. Relationship was assigned according to the maximum LR value observed among the tested relationships.

3.3.3 Data collection

We collected video data from June to November 2018 on a daily basis spanning morning and afternoon (7:00 am until 5:00 pm). The videos were recorded by two operators (E.C., M.C.) and a field assistant via Panasonic HC-V380/V180 and Sony HDR-PJ240E cameras. In total, 224 videos were collected corresponding to 43.0 hours of video observation (mean hour/subject 4.84 ± 1.85 SD). During the video recording, we maintained a wide zoom to improve the data collection (e.g. recording more conflicts that occurred concomitantly). The videos were then analyzed, frame-by-frame when necessary, via freeware VLC 3.0.6 and extension Jump-to-Time. Before starting the systematic video analysis I.N. and G.C. supervised M.C. and E.C. in a training period of 24 h to reach an interobserver reliability score (Cohen's k) of at least 0.81 for aggression, post-conflict affiliation, and anxiety-related behaviors (strong agreement *sensu* McHugh 2012). The Cohen's k value was measured using the R function "cohen.cappa" and libraries "irr" and "psych" (R version 3.5.3). From the video analysis, we extracted 216 aggressive events including 104 pigs. However, not all pigs engaged in conflicts or were present in post-conflict contexts (plus in different cases the victim or aggressor or both the opponents could move out of sight) or in all situations (reconciliation, TUC and TSC). Moreover, eight out of 104 pigs were culled

during the study period (see *The study group*) and for these animals we did not have data in all contexts/situations. For all these reasons, the sample sizes of the different analyses are different.

3.3.4 Operational definitions

For each conflict, we recorded the identity and features (e.g. gender, age, kinship) of the aggressor (i.e. the initiator of the conflict), victim (i.e. the aggression's recipient) and third-party or bystander (i.e. a pig not involved in the conflict that at the end of the aggression engaged in an affiliative contact with one of the former opponents). For data analyses, dyads were classified as weakly related (1st or 2nd cousins and unrelated individuals) and closely related (half- and full-siblings). The triadic affiliation was distinguished in solicited (TSC, Triadic Solicited Contact) if started by the victim/aggressor and unsolicited (TUC, Triadic Unsolicited Contact) if started by the third party (Fraser and Aureli 2008; Fraser et al. 2009).

To evaluate the occurrence of reconciliation (*Prediction 1a*), TSC (*Prediction 2a*) and TUC (*Prediction 3a*), with either victim or aggressor we employed the standard PC-MC method used in post-conflict studies on animals (de Waal and Yoshihara 1983; Arnold and Aureli 2010). After each agonistic event, we followed the opponents for a 3-min Post-Conflict period (PC). We used a 3-min time window as it has been previously demonstrated in the same study group that anxiety-related behaviors dropped within such time window (Norscia et al. 2021b). For each PC, a corresponding 3-min Matched Control observation (MC) of the behavior of the same individuals is recorded. This observation is usually carried out on the next possible day at the same time, and in the same social (presence of at least four individuals other than the opponents within max 20 m) and environmental context (same weather and time ± 1 h) on the original victim and aggressor, in absence of conflict in the previous 10 min. For both PC and MC, we recorded (i) the time gap (measured as mm:ss,00) between the starting of the PC or MC and the occurrence of the first affiliative contact (if present) between victim-aggressor, bystander-victim and bystander-aggressor, (ii) the type of first affiliative contact (the post-conflict affiliative contacts recorded were instantaneous events *sensu* Altmann 1974, such as nose-nose or nose-body contacts), and (iii) the initiator of affiliation. By this procedure, we obtained an equal number of PC and MC observation pairs that were compared with respect to the length of the time gap between the starting of PC/MC and the first affiliative contact between victim-aggressor, bystander-victim and bystander-aggressor. Pairs were classified as attracted - when the time-gap was shorter in PC than MC or affiliative contact was present only in PC-, dispersed - when the time-gap was shorter in MC than PC or affiliative contact was present only in MC-, and neutral - when the time-gap was equal in both PC and MC or affiliative contact did not occur in both PC and MC. In all our analyses we included pigs with at least 3 PC-MC pairs so that they could have at least one pair per type (attracted, dispersed and neutral pairs; Schino et al. 1998). As per de Waal and Yoshihara (1983), the presence of reconciliation and/or triadic affiliation can be confirmed if the number of attracted pairs is significantly higher than the number

of dispersed pairs at the individual level. We evaluated individual conciliatory levels by measuring the Corrected Conciliatory Tendency defined as ‘attracted minus dispersed pairs divided by the total number of PC-MC pairs’ (Veenema et al. 1994). Individual Corrected Conciliatory Tendencies were used to determine the mean group Corrected Conciliatory Tendency. We used the same formula to calculate the Triadic Contact Tendency for either solicited and unsolicited affiliation.

A previous report indicates that in the same study population the level of specific self-directed behaviors (i.e. head/body shaking, vacuum-chewing, yawning, and scratching/body rubbing) can be used as reliable anxiety indicators (Norscia et al. 2021b). Using such indicators, we investigated if, in absence of previous reconciliation, triadic affiliation could reduce anxiety levels in the study subjects. In particular, we compared the levels of anxiety-related behaviors of aggressor, victim and (for TUCs toward the victim) third parties, in the following three post-conflict conditions: (1) no triadic affiliation; (2) either solicited (*Self Anxiety Reduction Hypothesis, Prediction 2c*) or unsolicited contacts (*Appeasement Hypothesis and Consolation Hypothesis, Prediction 3e*); and (3) matched-control condition (MC; absence of aggression). We considered individuals that acted as victim, aggressor or third party in at least three aggressive events. Moreover, we considered the aggressive events in which only one type of triadic affiliation (i.e. solicited or not solicited) with either victim or aggressor occurred. During post-conflict time-window (3-min), we evaluated the numbers of target behaviors before and after the triadic affiliation and then we normalized these numbers over the min of observation before and after the affiliation.

3.3.5 Statistical analyses

In the case of non-normal distributions of data (Kolmogorov–Smirnov test: $p < 0.05$) we used non-parametric statistics for the analyses (Siegel and Castellan 1988). In particular, we applied the Wilcoxon’s signed rank test corrected for ties for two dependent samples to verify: (i) the occurrence of reconciliation (*Prediction 1a*), TSC (*Prediction 2a*) and TUC (*Prediction 3a*) by comparing the numbers of attracted vs dispersed pairs at the individual level; (ii) if kinship could affect the level of reconciliation (*Prediction 1b*), TSCs (*Prediction 2b*) and TUCs (*Prediction 3b*) by comparing the individual proportion of conciliatory/triadic contacts with weakly-related vs closely-related companions over the total contacts; (iii) the *Victim Protection Hypothesis* for both solicited and unsolicited triadic contacts (*Predictions 2d and 3d*) by comparing the hourly frequency of renewed attacks directed to the victim after vs no occurrence of either TSC or TUC; (iv) if TUCs could reduce the probability of renewed attacks by the aggressor on other group members (*Appeasement Hypothesis, Prediction 3c*), by comparing the number of attacks in presence vs absence of TUC. Via the non-parametric Friedman test for k-dependent samples, we compared the hourly frequencies of aggressor/victim/third-party anxiety-related behaviors across conditions (PC_{NOTri}, PC_{YESsol}/PC_{YESuns}, MC; *Predictions*

2c and 3e). The Bonferroni-Dunn *post-hoc* test was used for post-hoc pairwise comparisons.

Owing to a normal distribution (Kolmogorov–Smirnov test: $P \geq 0.05$), we used the parametric paired t-test for two dependent samples to compare the proportion of first post-conflict affiliative contacts initiated by victims vs aggressors over the total contacts.

For all the analyses evaluating the occurrence and the possible effects of TSC and TUC, we have considered only those triadic contact events (solicited and unsolicited) that occurred before or in absence of reconciliation.

3.4 Results

3.4.1 Prediction 1: Reconciliation

Prediction 1a. We found that the frequency of attracted pairs was significantly higher than that of dispersed pairs (Wilcoxon test $N_{\text{victims}} = 37$, $T = 0$, ties = 21, $p < 0.001$). This result confirms the occurrence of reconciliation in the study group of domestic pigs.

Prediction 1b. The individual proportion of conciliatory contacts was higher with weakly related than closely related fellows (Wilcoxon test $N_{\text{victims}} = 21$, $T = 1$, ties = 1, $p < 0.001$; **Figure 2**). Hence, in the study pigs reconciliation did not comply with the *Valuable Relationship Hypothesis* (see Introduction) because it was more frequent between weakly than closely related opponents.

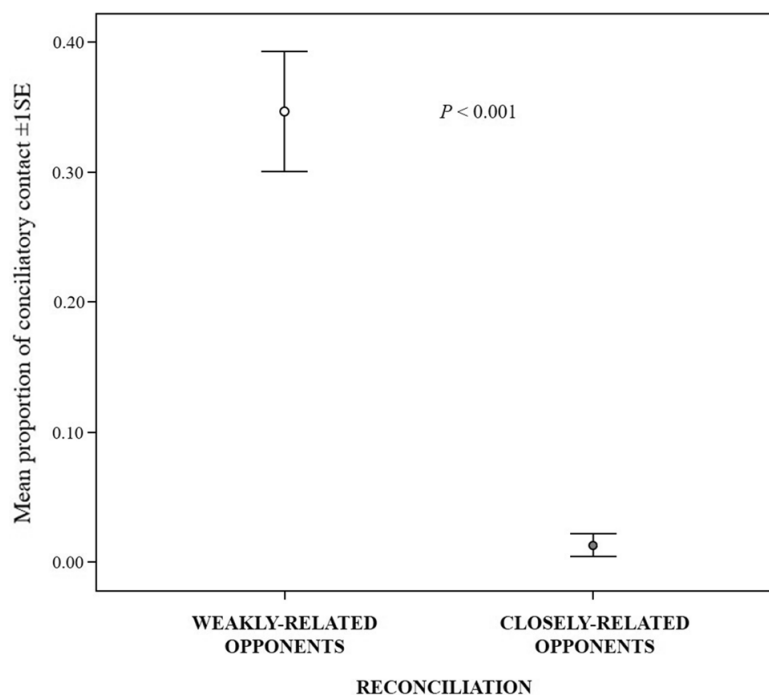


Figure 2. Error bars representing mean proportion of conciliatory contact ($\pm 1SE$) between weakly-related and closely-related victim- aggressor dyads.

Aggressor and victim started the first affiliative conciliatory contact at comparable levels (Paired t-test $N_{\text{individuals}} = 15$, $df = 14$, $t = -1.102$, $p = 0.289$; mean affiliations $\pm$ SE performed as aggressor 0.14 ± 0.04 and as victim 0.20 ± 0.07).

3.4.2 Prediction 2: Triadic solicited contacts (TSC)

Prediction 2a. When considering the affiliative contacts directed by victims towards third parties, we found that the attracted pairs were significantly higher than dispersed pairs (Wilcoxon test $N_{\text{victims}} = 35$, $T = 34$, ties = 11, $p = 0.001$). Hence, this result confirms the occurrence of triadic post-conflict affiliative contacts solicited by the victim (TSC) in the study group. However, 95.2% of affiliative contacts started by victims were not reciprocated by third parties (mean $\pm$ SE: not exchanged affiliation 0.95 ± 0.09 ; exchanged affiliation 0.05 ± 0.03). When considering affiliative contacts directed by aggressors towards third parties, we found that the attracted pairs did not significantly differ from the dispersed pairs (Wilcoxon test $N_{\text{aggressors}} = 30$, $T = 83.5$, ties = 9, $p = 0.245$). Hence, we cannot confirm the occurrence of triadic post-conflict affiliative contacts solicited by the aggressor in the study group. Consequently, *Predictions 2b*, *2c* and *2d* could only be tested on the TSC between victim and third party.

Prediction 2b. The proportion of TSCs was higher with closely related than weakly related third parties (Wilcoxon test TSC: $N_{\text{victims}} = 43$, $T = 189$, tie = 3, $p = 0.003$; **Figure 3**). This finding shows that victims directed their affiliative contacts more frequently towards closely than weakly related third parties.

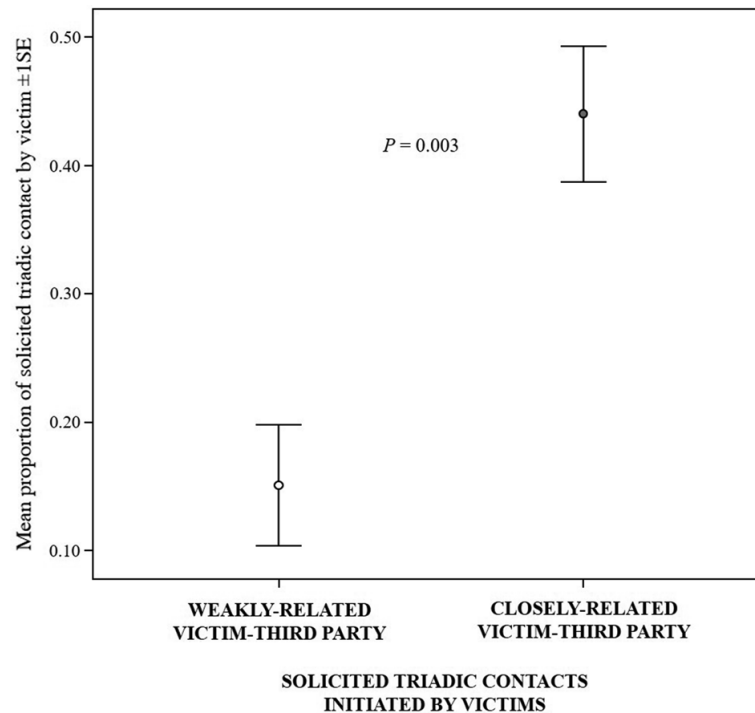


Figure 3. Error bars representing mean proportion of solicited triadic contact ($\pm$ 1SE) initiated by the victim and directed towards weakly- related and closely-related third party.

Prediction 2c. The levels of victim's anxiety-related behaviors significantly differed across the three conditions MC, PC without triadic affiliation and PC with only solicited triadic affiliation (Friedman test $N_{\text{victims}} = 30$, $\chi^2 = 14.026$, $df = 2$, $p = 0.001$; **Figure 4**). Particularly, the pairwise comparisons revealed a significant difference between MC-PC without triadic affiliation and MC-PC with only solicited affiliation (Bonferroni-Dunn post-hoc test; MC < PC without affiliation: $Q = 0.667$; $p = 0.029$; MC < PC with affiliation: $Q = 0.683$; $p = 0.024$) but not between PC without triadic affiliation and PC with only solicited affiliation ($Q = -0.017$; $p = 1.000$). Thus, the levels of victim's anxiety increased after a conflict but did not significantly decrease after the solicited triadic affiliation.

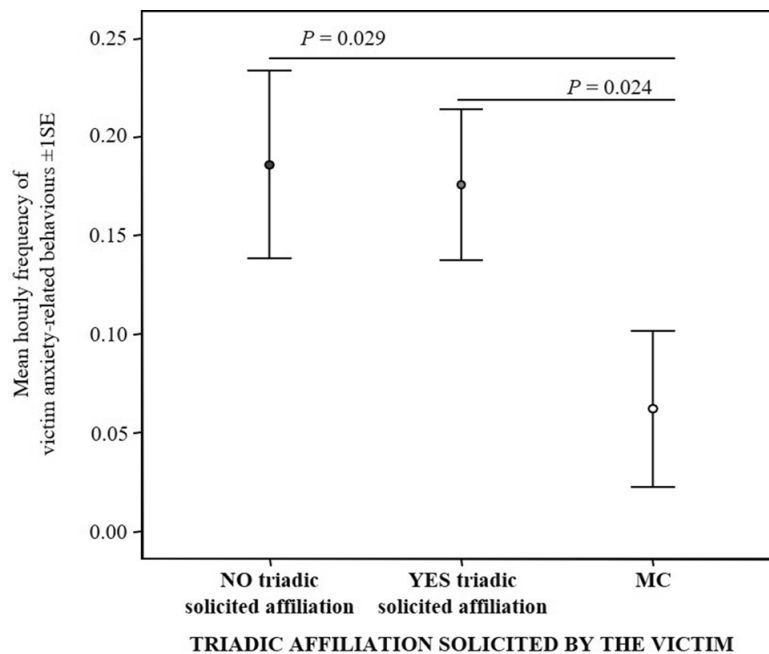


Figure 4. Error bars representing mean hourly frequency ($\pm 1SE$) of victim's anxiety-related behaviors under three conditions: post-conflict period with the absence of solicited contacts initiated by the victim towards a third party (NO triadic solicited affiliation), post-conflict period with the presence of solicited contacts initiated by the victim towards a third party (YES triadic solicited affiliation) and, control period not preceded by any aggression (MC).

Prediction 2d. The levels of further aggression against the victim by the aggressor were not reduced following TSCs (Wilcoxon test $N_{\text{victims}} = 31$, $T = 19.5$, ties = 21, $p = 0.410$). Thus, TSCs did not have a protecting effect towards the victim against further attacks by the aggressor.

3.4.3 Prediction 3: Triadic unsolicited contacts (TUC)

Prediction 3a. When considering the affiliative contacts directed by third parties towards victims (TUC), we found that the attracted pairs were significantly higher than dispersed pairs (Wilcoxon test $N_{\text{victims}} = 41$, $T = 15$, ties = 25, $p = 0.003$). When

considering the affiliative contacts directed by third parties towards aggressor, we found that the attracted pairs were significantly higher than dispersed pairs (Wilcoxon test $N_{\text{aggressors}} = 31$, $T = 7.5$, ties = 14, $p < 0.001$). This finding confirms the occurrence of TUCs directed by third parties towards both victims and aggressors in the study group.

Prediction 3b. Close-kin engaged in a higher proportion of TUCs than distant-kin toward either the victim (Wilcoxon test $N_{\text{victims}} = 15$, $T = 2$, ties = 0, $p < 0.001$; **Figure 5**) or the aggressor ($N_{\text{aggressors}} = 18$, $T = 0$, ties = 2, $p < 0.001$; **Figure 5**). Hence, third parties offered affiliative contacts more frequently towards a victim or an aggressor that was closely rather than weakly related with them.

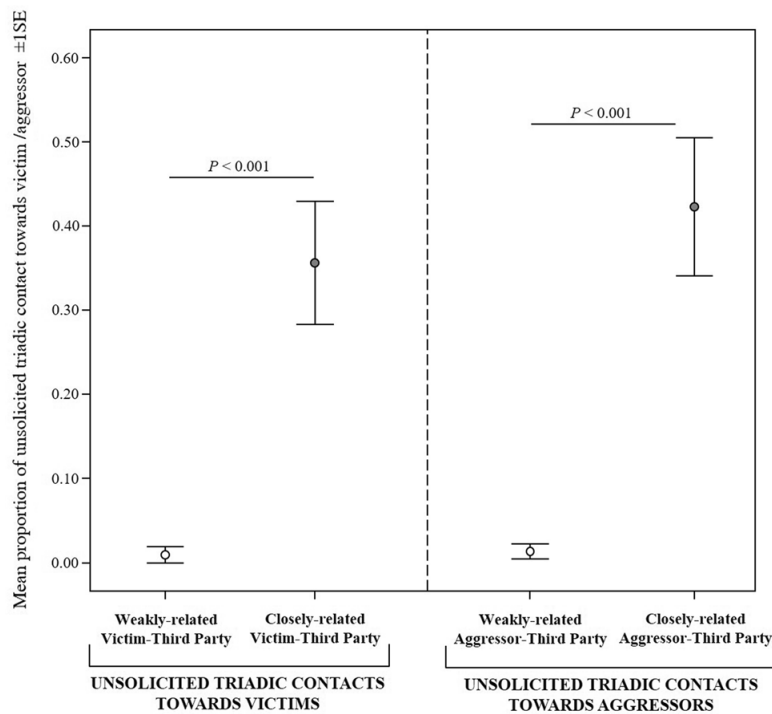


Figure 5. Error bars representing mean proportion of unsolicited triadic contact ($\pm 1SE$) initiated by a third party and directed (i) (on the left part of the graph) towards weakly related and closely related victims and (ii) (on the right part of the graph) towards weakly related and closely related aggressors.

Predictions 3c. The levels of renewed attacks by the aggressor towards other group members and the victim were lower in presence than in absence of TUCs exchanged with the aggressor (Wilcoxon test $N_{\text{aggressors}} = 13$, $T = 1$, ties = 4, $p = 0.008$). Hence, TUCs involving aggressors reduced the risk of renewed aggression, thus possibly having a calming effect.

Prediction 3d. The levels of further aggression against the victim by the previous aggressor were comparable in the presence and absence of TUCs exchanged with the victim (Wilcoxon test $N_{\text{victims}} = 13$, $T = 0$, ties = 9, $p = 0.125$). This result did not confirm the protecting effect of TUCs directed towards the victim.

Prediction 3e. In the case of TUCs towards the aggressor, the levels of aggressor anxiety-related behaviors significantly differed across the three conditions

MC, PC without triadic affiliation and PC with only triadic unsolicited affiliation (Friedman test $N_{\text{aggressors}} = 26$, $\chi^2 = 11.841$, $df = 2$, $p = 0.003$; **Figure 6a**). There was a significant difference between MC-PC without triadic affiliation (MC < PC without affiliation, Bonferroni-Dunn post-hoc test: $Q = 0.731$; $p = 0.025$) and no difference between MC- PC with only unsolicited affiliation and PC without triadic affiliation- PC with only unsolicited affiliation (MC-PC with affiliation: $Q = 0.481$; $p = 0.249$; PC without affiliation-PC with affiliation: $Q = 0.250$; $p = 1.000$). These findings indicate that the levels of anxiety in the aggressor increased after the conflict but did not significantly decrease after TUCs.

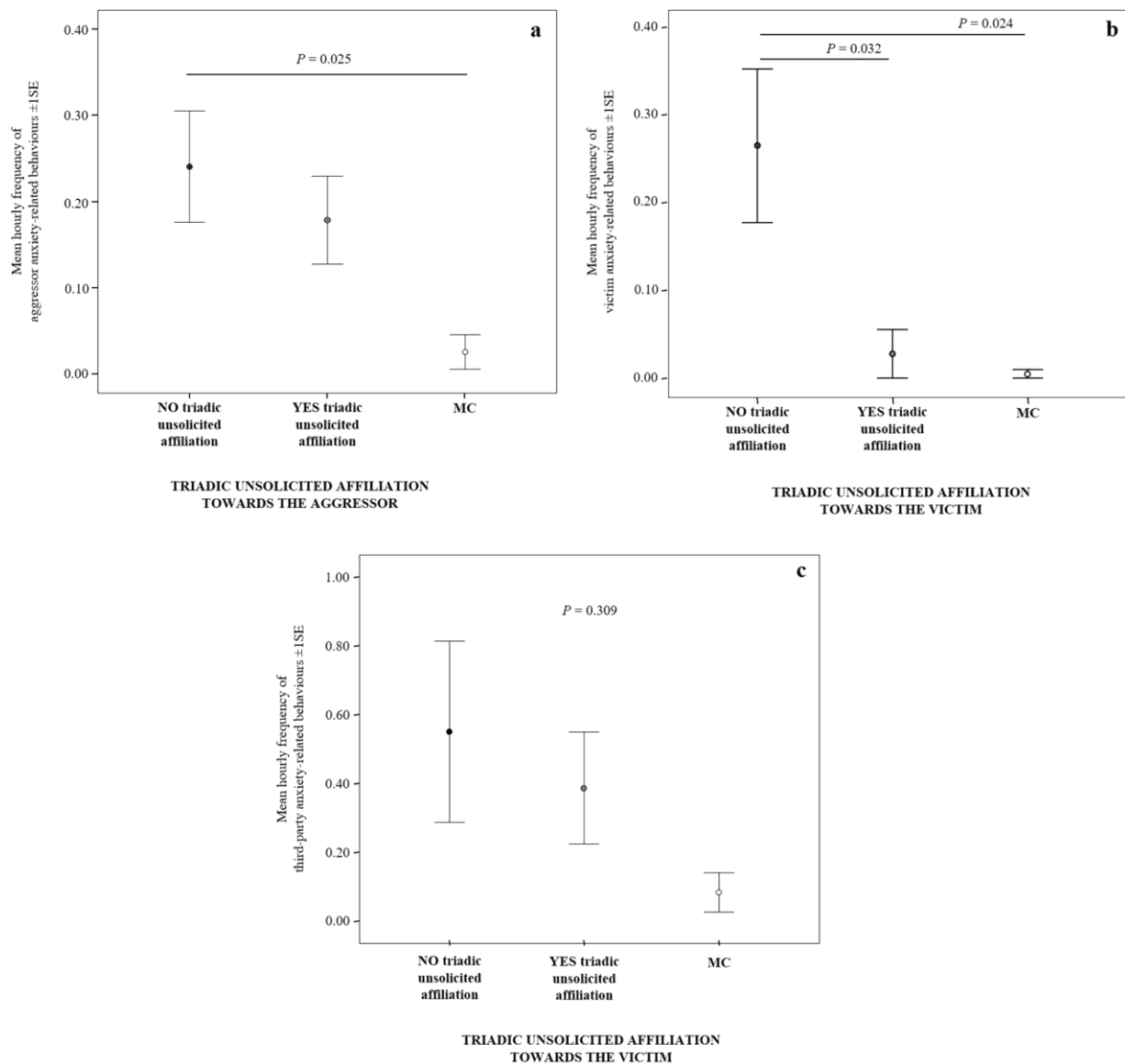


Figure 6. Error bars representing mean hourly frequency ($\pm 1SE$) of (a) aggressor, (b) victim and (c) third-party anxiety-related behaviors under three conditions: post-conflict (PC) period with absence (NO) of unsolicited contacts directed to the aggressor (a) or to the victim (b, c), post-conflict period with presence (YES) of unsolicited con- tacts directed to the aggressor (a) or to the victim (b, c), and control period (MC) not preceded by any aggression.

In the case of TUCs towards the victim, the levels of victim anxiety-related behaviors significantly differed across the three conditions (Friedman test $N_{\text{victims}} = 12$, $\chi^2 = 15.500$, $df = 2$, $p < 0.001$; **Figure 6b**). The pairwise comparisons revealed a significant difference between MC-PC without triadic affiliation and PC without triadic affiliation-PC with only triadic unsolicited affiliation (Bonferroni-Dunn post-hoc test; MC < PC without affiliation: $Q = 1.083$; $p = 0.024$; PC without affiliation > PC with affiliation: $Q = 1.042$; $p = 0.032$) but not between MC- PC with only unsolicited affiliation ($Q = 0.042$; $p = 1.000$). As a control, we checked for the variation in the levels of anxiety-related behaviors in the third parties affiliating with victims across the same conditions and we found no significant difference (Friedman test $N_{\text{third-parties}} = 10$, $\chi^2 = 2.348$, $df = 2$, $p = 0.309$; **Figure 6c**). Hence, TUCs directed to the victim had a consolatory effect on the victim itself (i.e. a decreased of anxiety-related behaviors). However, TUCs did not reduce the anxiety levels in the third party, thus not showing a self-distress reduction effect.

All results are summarized in **Table 1** and the post-conflict strategies found are outlined in **Figure 1**.

Table 1. Summary of results obtained in relation to different post-conflict behaviours showed by domestic pigs, associated with cognitive substrates and tested hypotheses

Post-conflict strategy	Individual mean $\pm$ SD number of PC-MC pairs	Involved participants	Social bias	Cognitive substrates	Tested Hypotheses
Reconciliation PC-MC=149 CCT = 13.6% ± 3.0 SE	4.03 $\pm$ 1.61	AG & VC	Yes, preferentially between weakly-related individuals	Second subject participatory capabilities (individual recognition and implicit memory of previously encountered subjects); attributing $\neq$ social value to others	<i>Valuable Relationship Hypothesis</i> rejected
Triadic contacts	4.50 $\pm$ 2.05	TSC by AG PC-MC = 134	*	*	*

	4.03 ±1.60	TSC by VC	Yes, preferentially between closely-related individuals	Intrinsic regulatory mechanisms (change in own experience)	<i>Self-Anxiety Reduction Hypothesis</i> rejected
		PC-MC =141 TCT _{sol} = 20.1% ±5.0 S.E.			<i>Victim Protection Hypothesis</i> rejected
	3.70 ±1.00	TUC toward AG by TP	Yes, preferentially between closely-related individuals	Extrinsic regulatory mechanisms (change in others' experience)	<i>Appeasement Hypothesis</i> supported
		PC-MC =114 TCT _{uns} = 15.9% ±3.6 S.E.			
	3.50 ±1.30	TUC toward VC by TP		More elements of MNS/PAM based social appraisal	<i>Victim Protection Hypothesis</i> rejected
		PC-MC =144 TCT _{uns} = 14.3% ±3.4 S.E			<i>Consolation Hypothesis</i> supported

Legend: AG = Aggressor, VC= Victim, TP = Third-Party, PC-MC = Post-Conflict/Matched Control pairs, CCT = Corrected Conciliatory Tendency (mean group value), TSC = Triadic Solicited Contact, TUS = Triadic Unsolicited Contact, TCT = Triadic Contact Tendency (mean group value), PAM = Perception-Action Model, MNS = Mirror Neuron System.

3.5 Discussion

Our results show that domestic pigs are able to adopt a wide array of post-conflict strategies (as per de Waal 2000). Compared to the Matched Control condition (absence of conflict), in the Post-Conflict condition it was more likely to observe affiliation between former opponents (i.e. reconciliation), between victim and a third party (both solicited - TSC - and unsolicited - TUC - triadic contacts), and between the aggressor and a third party (only TUC) (**Table 1**; **Figure 1**). The enactment of these post-conflict strategies, the different individuals that they involve and the effect that they produce inform the ability to attribute different social values to others, the presence of elements of intrinsic and extrinsic regulation, and possibly social appraisal (*sensu* Zaki and Williams 2013; Walle et al. 2017). These aspects are summarized in **Table 1** and detailed below.

Reconciliation. Reconciliation was present in the domestic pigs under study and was equally started by the aggressor or the victim (*Prediction 1a* supported). Moreover, reconciliation was not randomly distributed across dyads of opponents, even though not in the way it was expected. As a matter of fact, conciliatory contacts were highest between distant-kin rather than close-kin (*Prediction 1b* rejected). Hence, pigs possess the basic individual recognition and implicit memory skills that are necessary to engage in post-conflict reunion and attribute different social values to others. Reconciliation can be affected by the *value* and the *security* of the relationship between subjects (Cords and Aureli 2000). The *value* depends on the benefit that the subjects obtain in terms of affiliation, food-sharing, and social support. *Security* refers to the confidence that a subject has about the solidity of the relation and the predictability of the partner's behavioral response (Cords and Aureli 2000). The damage caused by conflict tends to be lower within highly predictable relationships (Koski 2015). Hence, if relationships are secure, the necessity of reconciling a previous conflict may decrease. In this respect, the relationship between closely related pigs can be highly secure, with little necessity for reconciliation. On the other hand, when a large number of animals live together (as it occurred in our study) weakly associated subjects can gain benefit from limiting their aggressiveness and increasing social tolerance with other in-group competitors for resource monopolization (Andersen et al. 2004; Estevez et al. 2007). In this view, weakly related pigs may strategically use reconciliation to improve the likelihood of tolerant cohabitation.

Triadic solicited affiliation (TSC). The pigs under study engaged in TSC, initiated by the victim but not by the aggressor (*Prediction 2a* partially supported). Aggressors - typically in a dominant position - may not gain immediate benefit from affiliating with a third subject after a conflict. The victim started TSCs most frequently with closely related third parties (*Prediction 2b* supported). This result suggests that domestic pigs may possess intrinsic regulation mechanisms, whose success also depends on their relationship with the contacted individual (Zaki and Williams 2013). In this respect, individuals (i.e. former opponents) would look for social contact to possibly regulate their own experience. Our result is consistent with other studies showing that TSCs can be most frequently exchanged between closely associated subjects (Aureli and Schaffner 2002; Fraser et al. 2008; McFarland and Majolo 2012). Because aggression can spread within social groups (social facilitation effect; Wilson 2000; Cordoni and Palagi 2008; Romero et al. 2009), it is probable that victims face less risks of receiving an aggressive reaction by approaching a third parties with which they share a valuable relationship. Intriguingly, TSCs had no influence in reducing post-conflict anxiety in the victim (*Self Anxiety Reduction Hypothesis; Prediction 2c* rejected) and in decreasing the probability of receiving further attacks by the previous aggressor (*Victim Protection Hypothesis; Prediction 2d* rejected). From the cognitive point of view, we can hypothesize that TSCs do not require that third parties perceive and actively respond to the distress of others (former opponents) after activating shared representations, as foreseen when third parties show agency and

start prosocial behaviors (Decety et al. 2016). In our study, 95.2% of post-conflict affiliative contacts performed by victims were not unreciprocated by third parties. Hence, solicited affiliative contacts were passively accepted by the third party but victims did not actually receive any affiliation from the third party, which sets a marked difference from unsolicited contacts. This situation may explain why TSCs did not lead to successful social regulation. Importantly, this observation opens the question of whether unilateral, unreciprocated contacts can be considered as actual TSCs. This issue has not been tackled by previous literature - which generically refers to the occurrence of affiliative contacts - and should be addressed by future studies.

Triadic unsolicited affiliation (TUC). The pigs under study showed TUC offered by third parties to either the victim or the aggressor (*Prediction 3a* supported). Hence, based on the cognitive hypotheses, third parties might be able to adopt a non-egocentric perspective and - by shifting from the dyadic to the triadic level - individuals might be able to implicitly share the experiences of others and be attuned to their internal states (Ferrari and Gallese 2007; Rochat et al. 2009; Aaltola 2013; Seyfarth and Cheney 2015). As observed in other social species (Koski and Sterck 2007; Palagi et al. 2008; Palagi and Cordoni 2009; Fraser and Bugnyar 2010; Romero et al. 2010, 2011), TUCs in pigs were more frequently offered to victim or aggressor by close-kin (*Prediction 3b* supported, **Table 1**). This suggests that a mechanism of social regulation be in place, as the effectiveness of such regulation depends on the interacting subjects (Zaki and Williams 2013). Indeed, depending on the contacted individual (aggressor or victim), TUCs led to different social regulation effects. When TUCs were directed toward the aggressor, they possibly regulated third party's own experience (intrinsic regulation), as TUCs were followed by a reduction in the levels of renewed attacks toward group members (as per the *Appeasement Hypothesis*; *Prediction 3c* supported). No such reduction was observed when TUCs were offered to the victim (contrary to the *Victim Protection Hypothesis*; *Prediction 3d* rejected). On the other hand, when TUCs were offered to the victim, they were followed by an anxiety level decrease in the victim, which suggests possible extrinsic regulation (*Consolation Hypothesis* Fujisawa et al. 2006; Fraser et al. 2008) *Prediction 3e* partially supported. No anxiety decrease was observed in the aggressors when TUCs were offered to them.

Previous studies on non-human primates have shown that the reduction of the aggressor's aggressiveness does not necessarily imply the reduction of aggressor's anxiety (Romero et al. 2009, 2011). Our result suggests that in pigs via TUCs towards aggressors third parties can protect themselves from potential retaliation with a possible benefit for their direct fitness (Cords and Aureli 2000; Schino and Marini 2012).

TUCs toward the victim were followed by a reduction of the victim's anxiety, which suggests the possible presence of elements of social appraisal in pigs. Indeed, it has been hypothesized that social appraisal allows individuals to assess the valence of a social interaction (e.g. aggression) in which they are not directly involved by

detecting the emotional reaction (i.e. distress) of individuals involved in such interaction (Cordoni and Palagi 2008; de Waal and Preston 2017; Walle et al. 2017; Pérez-Manrique and Gomila 2018). As a consequence, the third party may restore the victim emotional homeostasis via affiliation. TUCs in pigs may have a consolatory function, which by definition comes into play when the triadic contacts work in reducing the victim's arousal (Fraser et al. 2008; Fraser and Bugnyar 2010; de Waal and Preston 2017). As hypothesized by the neurocognitive model of emotional contagion in humans, the third party behavior may lead to the reduction of the discrepancy between the third party (agent) and the other's internal state (Prochazkova and Kret 2017). An additional element is that anxiety-related behaviors decreased in the victims but not in the third parties affiliating with the victims, which suggests that TUCs might be other- more than self-oriented. However, triadic contacts were mostly directed to close-kin. Thus, they cannot be considered as properly altruistic (*sensu* Silk and House 2016) as they can lead to indirect kinship benefits.

In sum, this study showed that pigs possess different post-conflict strategies (*sensu* de Waal and van Roosmaleen 1979), including reconciliation and unsolicited triadic contacts. Because the affiliative contacts offered by the victim to a third party remained largely unreciprocated, the actual presence of solicited triadic contact is questionable and future studies should take the reciprocation issue into account. The observation that all the post-conflict strategies were skewed by kinship (and not randomly distributed across dyads) and that the function of unsolicited third-party contacts (renewed aggression probability or anxiety reduction) depended on the contacted individual, point toward complex cognitive abilities, spanning intrinsic/extrinsic socio-emotional regulation and elements of social appraisal. Future study replications - aimed at increasing the generalization of the findings to the species level - should take into account the following main *Constraints on Generality* (*sensu* Simons et al. 2017): (i) only adults were part of the target population, (ii) only the domestic form of *Sus scrofa* (and not the wild counterpart) could be investigated, (iii) group composition slightly changed over time (due to moderate culling) and (iv) males were castrated, which can decrease aggression frequency and/or intensity, and the actual animal sample available for split post-conflict analyses. Despite these limitations, this study has an overall good generalization potential because - due to the extremely low culling rates and high farming standards - data collection could last for over six months on a large mixed breed/sex group of animals ranging semi-freely in a natural habitat (that nonetheless ensured excellent observation conditions), with different kinship degree and an unexpectedly large age span (from early sexual maturity to almost two years of life). Although rarely found in pig farming (even when extensive), when available these conditions are essential to undertake further steps into the investigation of pig post-conflict phenomena and the understanding of their cognitive implications at the species level.

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Ethical Statement: Permissions and ethical evaluations were deemed not necessary under the Italian law Dlg. 4/2014 n. 26 that defines the guidelines for the use of animals in experimental and scientific research.

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3.6 References

- Aaltola, E. (2013). Empathy, intersubjectivity, and animal philosophy. *Environmental Philosophy*, 10(2), 75–96. <https://doi.org/10.5840/envirophil201310215>
- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, 49(3), 227–267. <https://doi.org/10.1163/156853974X00534>
- Andersen, I. L., Nævdal, E., Bakken, M., & Bøe, K. E. (2004). Aggression and group size in domesticated pigs, *Sus scrofa*: 'when the winner takes it all and the loser is standing small.' *Animal Behaviour*, 68(4), 965–975. <https://doi.org/10.1016/j.anbehav.2003.12.016>
- Arey, D. S., & Edwards, S. A. (1998). Factors influencing aggression between sows after mixing and the consequences for welfare and production. *Livestock Production Science*, 56(1), 61–70. [https://doi.org/10.1016/S0301-6226\(98\)00144-4](https://doi.org/10.1016/S0301-6226(98)00144-4)
- Arnold, K., & Aureli, F. (2010). Postconflict reconciliation. In C. J. Campbell, A. Fuentes, K. C. MacKinnon, S. C. Bearder, & R. M. Stumpf (Eds.), *Primates in Perspective* (2nd ed., pp. 608–625). Oxford University Press.
- Aureli, F. (1997). Postconflict anxiety in nonhuman primates: The mediating role of emotion in conflict resolution. *Aggressive Behavior*, 23(5), 315–328. [https://doi.org/10.1002/\(SICI\)1098-2337\(1997\)23:5%3C315::AID-AB2%3E3.0.CO;2-H](https://doi.org/10.1002/(SICI)1098-2337(1997)23:5%3C315::AID-AB2%3E3.0.CO;2-H)
- Aureli, F., & Schaffner, C. M. (2002). Relationship assessment through emotional mediation. *Behaviour*, 139(2–3), 393–420. <https://doi.org/10.1163/156853902760102726>
- Aureli, F., Cords, M., & Van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: a predictive framework. *Animal Behaviour*, 64, 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Byrne, R. W., & Whiten, A. W. (1988). *Machiavellian intelligence: social expertise and the evolution of intellect in monkeys, apes, and humans*. Clarendon Press, Oxford, England.
- Camerlink, I., & Turner, S. P. (2013). The pig's snout and its role in dominance relationships and harmful behaviour. *Applied Animal Behaviour Science*, 145, 84–91. <https://doi.org/10.1016/j.applanim.2013.02.008>
- Camerlink, I., Turner, S. P., Ursinus, W. W., Reimert, I., & Bolhuis, J. E. (2014). Aggression and affiliation during social conflict in pigs. *PLOS ONE*, 9(11), e113502. <https://doi.org/10.1371/journal.pone.0113502>
- Camerlink, I., Coulange, E., Farish, M., Baxter, E. M., & Turner, S. P. (2018). Facial expression as a potential measure of both intent and emotion. *Scientific Reports*, 8(1), 1–9. <https://doi.org/10.1038/s41598-018-35905-3>
- Caratti, S., et al. (2010). Analysis of 11 tetrameric STRs in wild boars for forensic purposes. *Forensic Science International: Genetics*, 4(5), 339–342. <https://doi.org/10.1016/j.fsigen.2010.07.001>

Caruana, F. (2019). The integration of emotional expression and experience: a pragmatist review of recent evidence from brain stimulation. *Emotion Review*, 11(1), 27–38.

Cordoni, G., & Norscia, I. (2014). Peace-Making in marsupials: the first study in the red-necked wallaby (*Macropus rufogriseus*). *PLOS ONE*, 9(1), e86859. <https://doi.org/10.1371/journal.pone.0086859>

Cordoni, G., & Palagi, E. (2008). Reconciliation in wolves (*Canis lupus*): new evidence for a comparative perspective. *Ethology*, 114, 298–308. <https://doi.org/10.1111/j.1439-0310.2008.01474.x>

Cordoni, G., & Palagi, E. (2015). Being a victim or an aggressor: different functions of triadic post-conflict interactions in wolves (*Canis lupus lupus*). *Aggressive Behavior*, 41(6), 526–536. <https://doi.org/10.1002/ab.21590>

Cordoni, G., Palagi, E., & Borgognini-Tarli, S. M. (2006). Reconciliation and consolation in captive western gorillas. *International Journal of Primatology*, 27, 1365–1382. <https://doi.org/10.1007/s10764-006-9078-4>

Cords, M., & Aureli, F. (2000). Reconciliation and relationship qualities. In F. Aureli & F. B. M. de Waal (Eds.), *Natural conflict resolution* (pp. 177–198). University of California Press.

Cords, M., & Thurnheer, S. (1993). Reconciliation with valuable partners by long-tailed macaques. *Ethology*, 93, 315–325. <https://doi.org/10.1111/j.1439-0310.1993.tb01212.x>

Croney, C. C., & Boysen, S. T. (2021). Acquisition of a joystick-operated video task by pigs (*Sus scrofa*). *Frontiers in Psychology*, 12, 631755. <https://doi.org/10.3389/fpsyg.2021.631755>

Dalmau, A., Martínez-Macipe, M., Manteca, X., & Mainau, E. (2020). Sex differences in group composition and habitat use of Iberian free-range pigs. *Frontiers in Veterinary Science*, 7, 600259. <https://doi.org/10.3389/fvets.2020.600259>

Das, M. (2000). Conflict management via third parties. In F. Aureli & F. B. M. de Waal (Eds.), *Natural conflict resolution* (pp. 263–280). University of California Press, Berkeley.

D'Eath, R. B. (2002). Individual aggressiveness measured in a resident-intruder test predicts the persistence of aggressive behaviour and weight gain of young pigs after mixing. *Applied Animal Behaviour Science*, 77(4), 267–283. [https://doi.org/10.1016/S0168-1591\(02\)00077-1](https://doi.org/10.1016/S0168-1591(02)00077-1)

D'Eath, R. B., & Turner, S. P. (2009). The natural behaviour of the pig. In J. N. Marchant-Forde (Ed.), *The welfare of pigs* (pp. 13–45). Springer, Dordrecht, Netherlands.

de Waal, F. B. M. (2000). Primates - a natural heritage of conflict resolution. *Science*, 289(5479), 586–590. <https://doi.org/10.1126/science.289.5479.586>

de Waal, F. B. M., & Aureli, F. (1996). Consolation, reconciliation, and a possible cognitive difference between macaques and chimpanzees. In A. E. Russon, K. A. Bard, & S. T. Parker (Eds.), *Reaching into Thought: The Minds of Great Apes* (pp. 80–110). Cambridge, UK: Cambridge University Press.

de Waal, F. B. M., & Aureli, F. (1997). Conflict resolution and distress alleviation in monkeys and apes. In C. S. Carter (Ed.), *The integrative neurobiology of affiliation* (pp. 317–328). New York Academy of Sciences.

de Waal, F. B. M., & Preston, S. D. (2017). Mammalian empathy: behavioural manifestations and neural basis. *Nature Reviews Neuroscience*, 18(8), 498. <https://doi.org/10.1038/nrn.2017.72>

de Waal, F. B. M., & van Roosmaleen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioral Ecology and Sociobiology*, 5, 55–66. <https://doi.org/10.1007/BF00302695>

de Waal, F. B. M., & Yoshihara, D. (1983). Reconciliation and redirected affection in rhesus monkeys. *Behaviour*, 85, 224–241. <https://doi.org/10.1163/156853983X00237>

de Jaegher, H., Di Paolo, E., & Gallagher, S. (2010). Can social interaction constitute social cognition? *Trends in Cognitive Sciences*, 14(10), 441–447. <https://doi.org/10.1016/j.tics.2010.06.009>

Decety, J., Bartal, I. B. A., Uzefovsky, F., & Knafo-Noam, A. (2016). Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150077. <https://doi.org/10.1098/rstb.2015.0077>

Dunbar, R. I. M., & Shultz, S. (2007). Evolution in the social brain. *Science*, 317(5843), 1344–1347. <https://doi.org/10.1126/science.1145463>

Estevez, I., Andersen, I. L., & Nævdal, E. (2007). Group size, density and social dynamics in farm animals. *Applied Animal Behaviour Science*, 103(3–4), 185–204. <https://doi.org/10.1016/j.applanim.2006.05.025>

Ferrari, P. F., & Gallese, V. (2007). Mirror neurons and intersubjectivity. *Advances in Consciousness Research*, 68, 73. <https://doi.org/10.1075/aicr.68.08fer>

Fraser, O. N., & Aureli, F. (2008). Reconciliation, consolation and postconflict behavioral specificity in chimpanzees. *American Journal of Primatology*, 70, 1–10. <https://doi.org/10.1002/ajp.20608>

Fraser, O. N., & Bugnyar, T. (2010). Do ravens show consolation? Responses to distress in others. *PLOS ONE*, 5(5), e10605. <https://doi.org/10.1371/journal.pone.0010605>

Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. *Proceedings of the National Academy of Sciences USA*, 105, 8557–8562. <https://doi.org/10.1073/pnas.0804141105>

Fraser, O. N., Koski, S. E., Wittig, R. M., & Aureli, F. (2009). Why are bystanders friendly to recipients of aggression? *Communicative & Integrative Biology*, 2(3), 285–291. <https://doi.org/10.4161/cib.2.3.8718>

Freiwald, W. A. (2020). Social interaction networks in the primate brain. *Current Opinion in Neurobiology*, 65, 49–58. <https://doi.org/10.1016/j.conb.2020.08.012>

Fujisawa, K. K., Kutsukake, N., & Hasegawa, T. (2006). Peacemaking and consolation in Japanese preschoolers witnessing peer aggression. *Journal of Comparative Psychology*, 120(1), 48–57. <https://doi.org/10.1037/0735-7036.120.1.58>

Gheusi, G., Bluthé, R. M., Goodall, G., & Dantzer, R. (1994). Social and individual recognition in rodents: methodological aspects and neurobiological bases. *Behavioral Processes*, 33, 59–87. [https://doi.org/10.1016/0376-6357\(94\)90060-4](https://doi.org/10.1016/0376-6357(94)90060-4)

Goumon, S., & Špinka, M. (2016). Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Animal Cognition*, 19(3), 501–511. <https://doi.org/10.1007/s10071-015-0950-5>

Goumon, S., Illmann, G., Leszkowová, I., Dostalová, A., & Cantor, M. (2020). Dyadic affiliative preferences in a stable group of domestic pigs. *Applied Animal Behaviour Science*, 230, 105045. <https://doi.org/10.1016/j.applanim.2020.105045>

Graves, H. B. (1984). Behavior and ecology of wild and feral swine (*Sus scrofa*). *Journal of Animal Science*, 58, 482–492. <https://doi.org/10.2527/jas1984.582482x>

Horback, K. (2014). Nosing around: play in pigs. *Animal Behavior and Cognition*, 1, 186–196.

Ikkatai, Y., Watanabe, S., & Izawa, E. I. (2016). Reconciliation and third-party affiliation in pair-bond budgerigars (*Melopsittacus undulatus*). *Behaviour*, 153(9–11), 1173–1193. <https://doi.org/10.1163/1568539X-00003388>

Jensen, P. (1982). An analysis of agonistic interaction patterns in group-housed dry sows - aggression regulation through an “avoidance order.” *Applied Animal Ethology*, 9(1), 47–61. [https://doi.org/10.1016/0304-3762\(82\)90165-1](https://doi.org/10.1016/0304-3762(82)90165-1)

Jensen, P. (1986). Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 16(2), 131–142. [https://doi.org/10.1016/0168-1591\(86\)90105-X](https://doi.org/10.1016/0168-1591(86)90105-X)

Jensen, P. (2002). Behaviour of pigs. In P. Jensen (Ed.), *The Ethology of Domestic Animals: An Introductory Text* (3rd ed., pp. 159–172). CAB International.

Kling, D., Tillmar, A. O., & Egeland, T. (2014). Familias 3: extensions and new functionality. *Forensic Science International: Genetics*, 13, 121–127. <https://doi.org/10.1016/j.fsigen.2014.07.004>

Koski, S. E. (2015). Reconciliation and peace-making: insights from studies on nonhuman animals. In *Interdisciplinary Search for Linked Resources*. <https://doi.org/10.1002/9781118900772.etrds0276>

Koski, S. E., & Sterck, E. H. M. (2007). Triadic postconflict affiliation in captive chimpanzees: does consolation console? *Animal Behaviour*, 73(1), 133–142. <https://doi.org/10.1016/j.anbehav.2006.04.009>

- Kristensen, H. H., Jones, R. B., Schofield, C. P., White, R. P., & Wathes, C. M. (2001). The use of olfactory and other cues for social recognition by juvenile pigs. *Applied Animal Behaviour Science*, 72(4), 321–333. [https://doi.org/10.1016/S0168-1591\(00\)00209-4](https://doi.org/10.1016/S0168-1591(00)00209-4)
- Leavens, D. A., Bard, K. A., & Hopkins, W. D. (2019). The mismeasure of ape social cognition. *Animal Cognition*, 22, 487–504. <https://doi.org/10.1007/s10071-017-1119-1>
- Marino, L., & Colvin, C. M. (2015). Thinking pigs: a comparative review of cognition, emotion, and personality in *Sus domesticus*. *International Journal of Comparative Psychology*, 28, 23859.
- Massen, J. J. (2017). Friendships in animals. In J. Vonk & T. K. Shackelford (Eds.), *Encyclopedia of Animal Cognition and Behavior* (pp. 1–6). Springer International Publishing.
- McFarland, R., & Majolo, B. (2011). Reconciliation and the costs of aggression in wild Barbary macaques (*Macaca sylvanus*): a test of the integrated hypothesis. *Ethology*, 117(10), 928–937. <https://doi.org/10.1111/j.1439-0310.2011.01948.x>
- McFarland, R., & Majolo, B. (2012). The occurrence and benefits of postconflict bystander affiliation in wild Barbary macaques. *Animal Behaviour*, 84(3), 583–591. <https://doi.org/10.1016/j.anbehav.2012.06.010>
- McHugh, M. L. (2012). Interrater reliability: the kappa statistic. *Biochemia Medica*, 22(3), 276–282.
- McLeman, M. A., Mendl, M., Jones, R. B., White, R., & Wathes, C. M. (2005). Discrimination of conspecifics by juvenile domestic pigs (*Sus scrofa*). *Animal Behaviour*, 70(2), 451–461. <https://doi.org/10.1016/j.anbehav.2004.11.013>
- Norring, M., Valros, A., Bergman, P., Marchant-Forde, J. N., & Heinonen, M. (2019). Body condition, live weight and success in agonistic encounters in mixed parity groups of sows during gestation. *Animals*, 13(2), 392–398. <https://doi.org/10.1017/S1751731118001453>
- Norscia, I., & Palagi, E. (2016). *The missing lemur link: An ancestral step in the evolution of human behaviour*. Cambridge University Press, Cambridge, UK.
- Norscia, I., Coco, E., Robino, C., Chierito, E., & Cordoni, G. (2021a). Yawn contagion in domestic pigs (*Sus scrofa*). *Scientific Reports*, 11, 1851. <https://doi.org/10.1038/s41598-020-80545-1>
- Norscia, I., Collarini, E., & Cordoni, G. (2021b). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>
- Palagi, E., & Cordoni, G. (2009). Postconflict third-party affiliation in *Canis lupus*: Do wolves share similarities with the great apes? *Animal Behaviour*, 78(4), 979–986. <https://doi.org/10.1016/j.anbehav.2009.07.017>

Palagi, E., Dall'Olio, S., Demuru, E., & Stanyon, R. (2014). Exploring the evolutionary foundations of empathy: Consolation in monkeys. *Evolution and Human Behavior*, 35, 341–349. <https://doi.org/10.1016/j.evolhumbeh>

Palagi, E., & Norscia, I. (2013). Bonobos protect and console friends and kin. *PLOS ONE*, 8(11), e79290. <https://doi.org/10.1371/journal.pone.0079290>

Palagi, E., Chiarugi, E., & Cordoni, G. (2008). Peaceful post-conflict interactions between aggressors and bystanders in captive lowland gorillas (*Gorilla gorilla gorilla*). *American Journal of Primatology*, 70, 949–955. <https://doi.org/10.1002/ajp.20587>

Palagi, E., Celeghin, A., Tamietto, M., Winkielman, P., & Norscia, I. (2020). The neuroethology of spontaneous mimicry and emotional contagion in human and non-human animals. *Neuroscience & Biobehavioral Reviews*, 111, 149–165. <https://doi.org/10.1016/j.neubiorev.2020.01.020>

Pallante, V., Stanyon, R., & Palagi, E. (2016). Agonistic support towards victims buffers aggression in geladas (*Theropithecus gelada*). *Behaviour*, 153(9–11), 1217–1243. <https://doi.org/10.1163/1568539X-00003369>

Pérez-Manrique, A., & Gomila, A. (2018). The comparative study of empathy: Sympathetic concern and empathic perspective-taking in non-human animals. *Biological Reviews*, 93, 248–269. <https://doi.org/10.1111/brv.12342>

Prochazkova, E., & Kret, M. E. (2017). Connecting minds and sharing emotions through mimicry: A neurocognitive model of emotional contagion. *Neuroscience & Biobehavioral Reviews*, 80, 99–114. <https://doi.org/10.1016/j.neubiorev.2017.05.013>

Puga-Gonzalez, I., Butovskaya, M., Thierry, B., & Hemelrijk, C. K. (2014). Empathy versus parsimony in understanding post-conflict affiliation in monkeys: Model and empirical data. *PLOS ONE*, 9(3), e91262. <https://doi.org/10.1371/journal.pone.0091262>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109, 42–50. <https://doi.org/10.1016/j.physbeh.2012.11.002>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology & Behavior*, 129, 221–229. <https://doi.org/10.1016/j.physbeh.2014.02.059>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: Emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, 18, 517–532. <https://doi.org/10.1007/s10071-014-0820-6>

Rochat, P., Passos-Ferreira, C., & Salem, P. (2009). Three levels of intersubjectivity in early development. In *Enacting Intersubjectivity: Paving the Way for a Dialogue Between Cognitive Science, Social Cognition, and Neuroscience* (pp. 173–190). University Svizzera Italiana, Switzerland, Lugano.

Romero, T., & de Waal, F. (2010). Chimpanzee (*Pan troglodytes*) consolation: Third-party identity as a window on possible function. *Journal of Comparative Psychology*, 124(3), 278–286. <https://doi.org/10.1037/a0019144>

Romero, T., Colmenares, F., & Aureli, F. (2009). Testing the function of reconciliation and third-party affiliation for aggressors in hamadryas baboons (*Papio hamadryas hamadryas*). *American Journal of Primatology*, 71(1), 60–69. <https://doi.org/10.1002/ajp.20619>

Romero, T., Castellanos, M. A., & de Waal, F. B. M. (2010). Consolation as a possible expression of sympathetic concern among chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*, 107(27), 12110–12115. <https://doi.org/10.1073/pnas.1006991107>

Romero, T., Castellanos, M. A., & de Waal, F. B. M. (2011). Post-conflict affiliation by chimpanzees with aggressors: Other-oriented versus selfish political strategy. *PLOS ONE*, 6, e22173. <https://doi.org/10.1371/journal.pone.0022173>

Schino, G., & Marini, C. (2012). Self-protective function of post-conflict bystander affiliation in mandrills. *PLOS ONE*, 7(6), e38936. <https://doi.org/10.1371/journal.pone.0038936>

Schino, G., & Sciarretta, M. (2015). Effects of aggression on interactions between uninvolved bystanders in mandrills. *Animal Behaviour*, 100, 16–21. <https://doi.org/10.1016/j.anbehav.2014.11.013>

Schino, G., Rosati, L., & Aureli, F. (1998). Intragroup variation in conciliatory tendencies in captive Japanese macaques. *Behaviour*, 135(7), 897–912. <https://doi.org/10.1163/156853998792640314>

Schütz-Bosbach, S., & Prinz, W. (2015). Mirrors match minds. In *New Frontiers in Mirror Neurons Research* (pp. 198–221). Ferrari, P. F., & Rizzolatti, G. (Eds.), Oxford University Press, Oxford, UK.

Seyfarth, R. M., & Cheney, D. L. (2015). Social cognition. *Animal Behaviour*, 103, 191–202. <https://doi.org/10.1016/j.anbehav.2015.01.030>

Siegel, S., & Castellan, N. J. (1988). *Nonparametric Statistics for the Behavioral Sciences* (2nd ed.). McGraw-Hill, New York.

Silk, J. B., & House, B. R. (2016). The evolution of altruistic social preferences in human groups. *Philosophical Transactions of the Royal Society B*, 371, 20150097. <https://doi.org/10.1098/rstb.2015.0097>

Sima, M. J., Matzinger, T., Bugnyar, T., & Pika, S. (2018). Reconciliation and third-party affiliation in carrion crows. *Ethology*, 124(1), 33–44. <https://doi.org/10.1111/eth.12699>

Simons, D. J., Shoda, Y., & Lindsay, S. (2017). Constraints on Generality (COG): A proposed addition to all empirical papers. *Perspectives on Psychological Science*, 12(6), 1123–1128. <https://doi.org/10.1177/1745691617708630>

Špinka, M. (2017). Behaviour of pigs. In *The Ethology of Domestic Animals: An Introductory Text* (3rd ed., pp. 214–227). P. Jensen (Ed.), Wallingford, UK: CAB International.

Stolba, A., & Wood-Gush, D. G. M. (1984). The identification of behavioural key features and their incorporation into a housing design for pigs. *Annales de Recherches Vétérinaires*, 15, 287–298. <https://doi.org/10.1017/S0003356100040411>

Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science*, 48(2), 419–425. <https://doi.org/10.1017/S0003356100040411>

Swallow, S. R., & Kuiper, N. A. (1988). Social comparison and negative self-evaluations: An application to depression. *Clinical Psychology Review*, 8(1), 55–76. [https://doi.org/10.1016/0272-7358\(88\)90049-9](https://doi.org/10.1016/0272-7358(88)90049-9)

Taylor, S. E., Neter, E., & Wayment, H. A. (1995). Self-evaluation processes. *Personality and Social Psychology Bulletin*, 21, 1278–1287.

Turner, S. P., Horgan, G. W., & Edwards, S. A. (2001). Effect of social group size on aggressive behaviour between unacquainted domestic pigs. *Applied Animal Behaviour Science*, 74(3), 203–215. [https://doi.org/10.1016/S0168-1591\(01\)00168-X](https://doi.org/10.1016/S0168-1591(01)00168-X)

Veenema, H. C., Das, M., & Aureli, F. (1994). Methodological improvements for the study of reconciliation. *Behavioural Processes*, 31, 29–38. [https://doi.org/10.1016/0376-6357\(94\)90035-3](https://doi.org/10.1016/0376-6357(94)90035-3)

Walle, E., Reschke, P., Camras, L., & Campos, J. (2017). Infant differential behavioral responding to discrete emotions. *Emotion*, 17, 1078–1091. <https://doi.org/10.1037/emo0000307>

Wilson, E. O. (2000). *Sociobiology: The New Synthesis*. The Belknap Press of Harvard University Press, Cambridge.

Wittig, R. M., & Boesch, C. (2005). How to repair relationships: Reconciliation in wild chimpanzees (*Pan troglodytes*). *Ethology*, 111, 736–763. <https://doi.org/10.1111/j.1439-0310.2005.01093.x>

Yorzinski, J. L. (2017). The cognitive basis of individual recognition. *Current Opinion in Behavioral Science*, 16, 53–57. <https://doi.org/10.1016/j.cobeha.2017.03.009>

Zaki, J., & Williams, W. C. (2013). Interpersonal emotion regulation. *Emotion*, 13(5), 803–810. <https://doi.org/10.1037/a0033839>

4. Witness for resolution: post-conflict quadratic affiliation in semi-free ranging pigs

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Abstract

In social mammals, post-conflict resolution can involve the reunion of former opponents (reconciliation), spontaneous/solicited post-conflict affiliation of a third party with either opponent (triadic contacts), and affiliation between other individuals (hereafter bystanders; quadratic contacts). Quadratic contacts - possibly informing complex cognitive abilities- have been neglected in post-conflict studies. We investigated quadratic affiliation in semi-free ranging pigs *Sus scrofa*, at the ethical farm Parva-Domus (Cavagnolo, Italy). Kinship was known. We collected behavioral data on adult pigs ($n = 104$) via video recordings (43 h) followed by video analyses. Affiliative and anxiety behaviors between bystanders were collected under post-conflict (PC; following a conflict between non-bystanders) and matched-control (MC; no conflict) conditions. Quadratic affiliation was present in pigs, as bystanders affiliated more in PC than MC, and such affiliation was followed by a decrease in the anxiety behaviors of both the interacting bystanders. Thus, quadratic contacts may be partly aimed at reducing one's own anxiety (intrinsic regulation). Quadratic affiliation was highest between closely related bystanders, which suggests that such affiliation may be most effective when close kin is involved. Quadratic affiliation was lowest after reconciliation and spontaneous triadic contacts. This suggests that direct peacemaking between opponents and spontaneous triadic contacts with close kin may most likely replace quadratic affiliation. Hence, pigs can be influenced by the negative events that affect other pigs - but not themselves - and their response may be modulated by social factors. Such non-random quadratic affiliation may point toward the presence of elements of social appraisal abilities in pigs.

4.1 Introduction

Group living bears benefits - such as decreased predation chances and increased access to food and breeding mates - but also costs, such as increased competition and inevitable conflict of interest over resources (de Waal 2000; Majolo et al. 2008; Chapman and Valenta 2015). When a conflict of interest emerges between two individuals and takes the form of aggression, the agonistic encounter can negatively affect the whole social group, as it can escalate and potentially lead to group disruption (De Marco et al. 2010; Schino and Sciarretta 2015). To reduce the possible damage deriving from aggression, different post-conflict strategies can be enacted and can involve the “peaceful” reunion of former opponents (*reconciliation*; de Waal and van Roosmalen 1979) or a “friendly” contact between either opponent and an uninvolved third party (*triadic affiliation*; Romero et al. 2009, 2011). In technical terms, reconciliation is defined as the first affiliative contact exchanged between the former opponents occurring in the first minutes following the end of the aggression (de Waal and van Roosmaleen 1979). Reconciliation likely requires individual recognition abilities and implicit memory of previously encountered subjects (Cords and Thurnheer 1993; Aureli et al. 2002) and - by restoring the relationship between former opponents and/or possibly facilitating access to resources - it can work in relieving the anxiety deriving from the conflict (Norscia and Cordoni 2014; Aureli 1997; Silk 2006; Romero et al. 2009; McFarland and Majolo 2011). Triadic affiliation is defined as the first affiliative contact exchanged between an uninvolved third party and either opponent in the first minutes after a conflict (Romero et al. 2009, 2011). Such triadic affiliation can be “*solicited*” if the affiliation with the third party is initiated by a former opponent (de Waal and Aureli 1996; de Waal 2000) or “*unsolicited*” if the third party takes agency in the post-conflict affiliation and spontaneously approaches and contacts one of the former opponents (de Waal and Preston 2017). Solicited affiliation may be involved in intrinsic social regulation (*sensu* Zaki and Williams 2013) as the former opponent initiates a social contact to possibly regulate *its own* experience (Cordoni et al. 2023). Indeed, solicited affiliation can decrease the probability to receive further aggression from other group members (Palagi and Norscia 2013; Palagi et al. 2014) and/or reduce self-anxiety (Palagi and Cordoni 2009; McFarland and Majolo 2012; Puga-Gonzalez et al. 2014). Unsolicited triadic affiliation may require that the individuals initiating it possess elements of social appraisal (*sensu* Walle et al. 2017) to possibly change their own experience and/or the experience of the contacted subject (intrinsic and/or extrinsic regulation; Cordoni et al. 2023). Unsolicited affiliation can indeed protect the aggression recipient from further attacks (Das 2000; Romero et al. 2011; Palagi and Norscia, 2013; Cordoni and Palagi 2015) and reduce its anxiety, in which case the functional term “consolation” is also used (de Waal and van Roosmaleen 1979; Fraser et al. 2008; Fraser and Bugnyar 2010; Romero and de Waal 2010; Palagi and Norscia 2013; Cordoni et al. 2023).

Overall, post-conflict affiliation can reduce the anxiety produced by the aggression in the opponents and/or third parties involved in such affiliation (e.g., de Waal and Aureli, 1997; Fraser et al. 2008, 2009; Fraser and Bugnyar 2010; Cordoni et

al. 2023). However, former opponents and third parties are not the only group members that can be affected by aggression because the effect of an aggressive event can reverberate on the entire group and its members, increasing social tension (De Marco et al. 2010). In this respect, the interactions between bystanders can form part of the conflict resolution process. In few studies on primates, it has been observed that uninvolved bystanders simply witnessing an aggression (not taking part as third parties in post-conflict strategies) can increase affiliation contacts between each other after the conflict (Judge and Mullen 2005; De Marco et al. 2010; Daniel and Alves 2015). This type of interaction between bystanders is called *quadratic affiliation*, which is more exactly defined as the first affiliative contact exchanged between bystanders in the first minutes after a conflict (Judge and Mullen 2005). To our knowledge, so far quadratic affiliation has been only reported in primates and specifically in three out of four African monkey species investigated for the presence of the phenomenon, namely Japanese macaques, (*Macaca fuscata*, Daniel and Alves 2015), hamadryas baboons (*Papio hamadryas*: Judge and Mullen 2005), and Tonkean macaques (*Macaca tonkeana*, De Marco et al. 2010) but not geladas (*Theropithecus gelada*, Leone et al. 2010). Interestingly, no quadratic affiliation has been detected in bottlenose dolphins *Tursiops truncatus*, which show both reconciliation and triadic contacts (Yamamoto et al. 2015, 2020). The absence of quadratic affiliation appears to be associated with tolerant species possessing relaxed dominance style, which would make conflicts not so stressful to induce affiliation between bystanders (Leone et al. 2010; Daniel and Alves 2015; Yamamoto et al. 2020).

When present, quadratic affiliation can help bystanders mitigate emotional arousal after witnessing a conflict (Judge and Mullen 2005). Indeed, in the few primate species where quadratic affiliation has been found, such affiliation worked in reducing self-directed behaviors associated to anxiety in the bystanders (hamadryas baboons, Judge and Mullen 2005; Japanese macaques, Daniel and Alves 2015), although not always consistently across groups (Tonkean macaques, De Marco et al. 2010). Hence, as it occurs with other post-conflict strategies, quadratic affiliation may contribute to restoring group homeostasis after a potentially disruptive event. Despite its relevance to group stability, quadratic affiliation has been almost totally neglected in animal post-conflict studies, possibly because it can prove difficult to reliably and comprehensively collect behavioral observations on opponents, third parties and bystanders altogether after a conflict and on a representative sample of agonistic encounters.

In this study, we investigated for the first time the possible occurrence of quadratic affiliation in domestic pigs *Sus scrofa* and specifically in a large mixed sex/breed group raised in a natural grassland-woodland habitat and under semi-free ranging conditions (extensive farming). In such conditions, pigs can express the full array of social behavioral patterns of their wild counterpart (i.e., wild boar; Jensen 1986; Jensen 2002; Stolba and Wood-Gush 1989; Norscia, Collarini et al. 2021). Domestic pigs are good candidates to investigate the phenomenon of quadratic affiliation because they show complex social interactions based on different sensory

modalities that include vocalizations and body postures used for inter-individual communication (d'Eath and Turner 2008; Horback 2014) and olfactory behaviors such as nose-to-body and nose-to-nose contacts that are used for social exploration and affiliation (Camerlink and Turner, 2013; Camerlink et al. 2014; Špinka 2017). Moreover, domestic pigs possess advanced cognition, including the ability to identify familiar individuals and items, different post-conflict strategies (reconciliation/triadic contacts), sensitivity to the emotional states of conspecifics, and ability to respond to their distress (Reimert et al. 2013; Marino and Colvin 2015; Reimert et al. 2015; Goumon and Špinka 2016; Camerlink et al. 2018; Norscia, Coco et al. 2021; Norscia, Collarini et al. 2021; Cordoni et al. 2023). Based on the previous framework we formulated the following predictions.

4.1.1 Prediction 1: Presence of quadratic affiliation

Pigs are not always tolerant as they can use repeated aggression to establish ranking positions especially during regrouping and females can form linear hierarchies (Meikle et al. 2010; D'Eath 2002; Andersen et al. 2004; Norring et al. 2019). Aggressive behavior in pigs can have important consequences on animal health as it can alter stress physiology and increase anxiety not only in the opponents but also in other group members (Fernandez et al. 1994; Norscia, Collarini et al. 2021). Moreover, Cordoni et al. (2023) found that pigs are able to engage in different post-conflict affiliation (reconciliation and triadic contacts) to restore group homeostasis. Hence, we expected to find an increase of affiliation between bystanders following a conflict compared to a control condition (*quadratic affiliation*).

4.1.2 Prediction 2 - Impact of quadratic affiliation on bystander anxiety

Previous studies have found that quadratic affiliation can work in reducing self-directed behaviors associated with anxiety in the bystanders (Japanese macaques, Daniel and Alves 2015; hamadryas baboons, Judge and Mullen 2005). Norscia et al. (2021) found that aggression can lead to an increase of anxiety/tension-related behaviors (self-scratching/body rubbing, head/body shaking, yawning, and vacuum chewing) and that the individuals that were not opponents - more than the opponents themselves - expressed the highest rates of such behaviors. Because in pigs conflicts can impact the whole group, we expected that quadratic affiliation would work in reducing anxiety in the bystanders (*Prediction 2a*). Moreover, in pigs spontaneous triadic post-conflict affiliation by third parties can decrease the anxiety in the recipient of an aggression and not in the third party (possibly informing elements of extrinsic emotional regulation; Cordoni et al. 2023). If the same applies to the affiliation between bystanders, we expect that the anxiety levels would be reduced in the contacted subject rather than in the individuals that initiate the affiliation (*Prediction 2b*).

4.1.3 Prediction 3 - Individual and social factors modulating quadratic affiliation

In pigs, different breeds can show different levels of aggressive social behavior (Breuer et al. 2003, Løvendahl et al. 2005; Turner et al. 2010) and social organizations of adults can vary depending on the sex, with males most commonly dispersing and females forming stable groups (Stolba and Wood-Gush 1989; d'Eath and Turner 2009; Podgórski, Lusseau et al. 2014; Podgórski Scandura et al. 2014). Despite these differences, we expected to find no differences in quadratic affiliation - if present - between sexes (*Prediction 3a*) and breeds (*Prediction 3b*) because these variables had no influence in other post-conflict behaviors and post-conflict anxiety levels (Norscia, Collarini et al. 2021; Cordoni et al. 2023).

Kinship can highly influence post-conflict behavior (e.g., Cheney and Seyfarth 1989). Previous studies on primates found that that quadratic affiliation did not occur more between kin bystanders (Judge and Mullen 2005; De Marco et al. 2010), even though in hamadryads baboons *Papio hamadryas* the kin of one opponent was more likely to affiliate with the kin of the other opponent (Judge and Mullen 2005). This result was interpreted as possible “quadratic reconciliation” between kin groups. Pig sociality - especially among females - is based on close kinship (Podgórski, Scandura et al. 2021) and a recent study (Cordoni et al. 2023) found that solicited and unsolicited post-conflict triadic contacts in pigs occurred mostly between former opponents and closely kin-related third parties. Hence - based on pig socio-biology - we expected that quadratic affiliation could be enhanced between kin bystanders and when opponents were closely related to bystanders (*Prediction 3c*).

Reconciliation can help repairing the relationship between former opponents and spontaneous affiliation by a third party can be more effective than solicited affiliation in reducing anxiety in the recipient of aggression, as it occurs in pigs and other species (Fraser et al. 2008; Fraser and Bugnyar 2010; Palagi and Norscia 2013; de Waal and Preston 2017; Cordoni et al. 2023). Third-party affiliation can be used as a substitute for reconciliation to restore group homeostasis after a conflict (Judge 1991; Fujisawa et al. 2006; Palagi and Cordoni 2009). Hence, we expected that quadratic affiliation - as a possible substitute of other post-conflict contacts - may be less frequent when reconciliation and spontaneous triadic contacts occurred after a conflict (*Prediction 3d*).

4.2 Materials and Methods

4.2.1 Study group and site

This study was carried out on 104 semi-free ranging adult pigs (7–22 months old) that lived in the same group (54 castrated males and 50 females of three mixed breeds (they were not pure breed because at least one grand-parent belonged to a different breed): Parma Black, Large White, and Piedmont Black). The pigs - individually marked every 4–7 days (during feeding when they grouped together)

depending on weather conditions via non-toxic livestock painting spray for individual recognition - were housed at the ethical farm “*Parva Domus*” (Cavagnolo, Turin – Italy) in a woodland natural area of about 13 ha (water available ad libitum; provision of food pellets once/day between 8:30 and 10:30 am: Ciclo Unico P, SILDAMIN®).

4.2.2 Kinship determination and genetical analyses

Kinship (close: full/half siblings; weak: others) was known from farmer notes and - for those cases in which kinship was unclear - kinship was confirmed via DNA analyses carried out at the Department of Public Health Sciences and Pediatrics, University of Torino (see Cordoni et al. 2023, for details). Genetic analyses were carried out on 31 pigs (two to three individuals sampled from different sibling generations). DNA was extracted by hair bulbs (gathered during the study period) via QIAmp DNA Investigator Kit (Qiagen; www.qiagen.com) by applying the provider’s protocol. 11 autosomic STRs were amplified via multiplex PCR Animal Type Pig PCR amplification kit (<http://www.biotype.de>; Biotype AG, Dresden, Germany). Capillary electrophoresis with SeqStudio system (Thermo Fisher Scientific; www.thermofisher.com) was used to obtain the genetic profile typing. Allele frequencies and kinship index (0.08) were based on a mixed sample of domestic pigs ($n = 412$), consisting of commercial lines commonly used in the production process (Caratti et al. 2010). The mutation rate for all markers was set at 0.002. For each possible dyad of pigs an unspecific kinship search was performed using Familias 3.1.5 “Blind Search” Module (Kling et al. 2014). Likelihood ratio (LR) was calculated for related individuals (sibling/half-siblings and 1st/2nd cousins), scaled versus unrelated. Relationship was assigned according to the maximum LR value observed among the tested relationships.

4.2.3 Data collection and operational definitions

In the period Jun-Nov 2018 two operators (E.C. and a field assistant) recorded videos (via Panasonic HC-V380/V180 and Sony HDR-PJ240E cameras; 224 videos; 43 h) on conflicts including at least one aggressive pattern (see Cordoni et al. 2023, for the full list and description; $N_{conflicts} = 86$), and during 3-min post-conflict (PC) and matched-control periods (MC; in absence of conflict). The videos were then analyzed, frame-by-frame or in slow motion, via freeware VLC 3.0.6 and extension Jump-to-Time. Intercoder reliability (Cohen’s k ; McHugh 2012) was at least 0.81 for aggression, post-conflict affiliation, and anxiety-related behaviors (strong agreement; Cordoni et al. 2023). The Cohen’s k value was measured using the R function “cohen.cappa” and libraries “irr” and “psych” (R version 3.5.3). Affiliation patterns extracted from videos included rest in contact, social touching, nose–nose contact, nose–body contact, head-over, and social rubbing (see Cordoni et al. 2023, for the full list and description). Aggressive patterns included lifting, biting, mounting, kicking, pushing, chasing, head-knocking (see Cordoni et al. 2023, for the full ethogram). We defined as 1) opponents: the two individuals directly engaging in a fight; 2) third parties: the individuals not

involved in the conflict that after the end of the aggression engaged in unsolicited or solicited affiliative contacts with one (or both) of the former opponents; 3) uninvolved bystanders (hereafter bystanders): the other individuals witnessing the aggression that were not involved in the conflict and in any post-conflict affiliation with either opponent (**Figure 1**). In the 3-min PC/MC we recorded data on: 1) identity, sex, and breed of opponents, third-parties and bystanders (initiating and receiving post-conflict affiliation); 2) if present, the first affiliative contact that occurred between former opponents (hereafter reconciliation), between a third party and either opponent (solicited and unsolicited triadic contacts), and between bystanders (possible quadratic contacts); 3) time of each of the affiliative contacts listed at the previous point; 4) anxiety-related behaviors (head/body shaking, scratching/body rubbing, yawning, vacuum chewing, as per Norscia, Collarini et al. 2021).

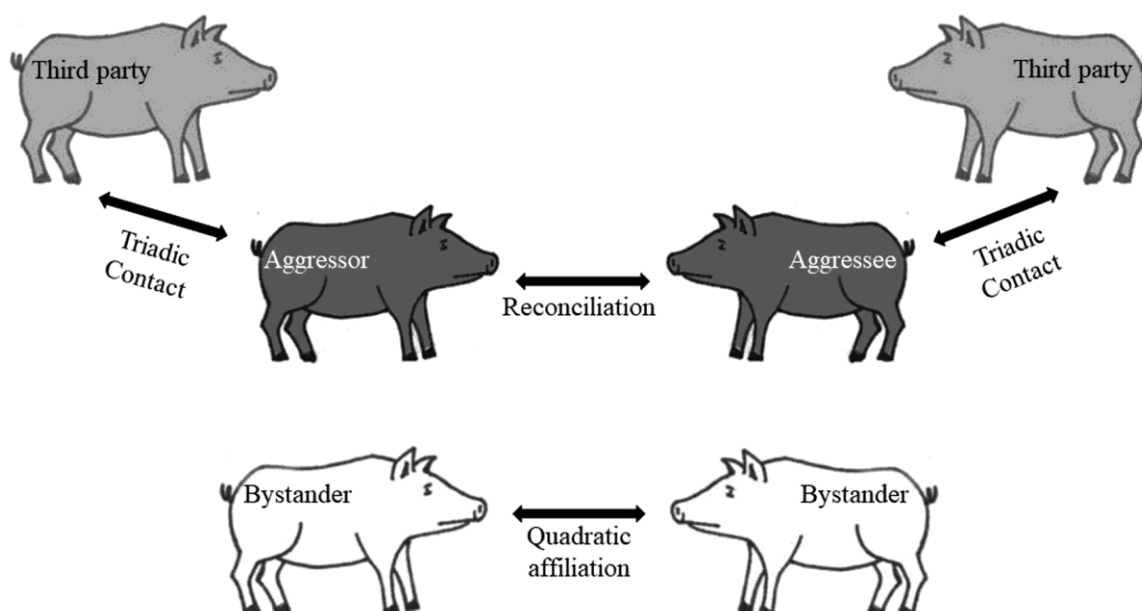


Figure 1. Quadratic contacts within all possible post-conflict affiliation dynamics. Third parties (light gray), bystanders (white) and opponents (aggressor and aggressee; dark gray) are indicated. For the purpose of this study, “bystanders” are those individuals that were not involved in the conflict and in any post-conflict affiliation with either opponent (quadratic contacts). Third parties indicate individuals that were not involved in the conflict but affiliated with either opponent after the conflict (triadic contacts). Opponents are indicated as aggressor and aggressee, which can also affiliate after a conflict (reconciliation).

A posteriori, we determined the degree of kinship between the bystanders involved in post-conflict affiliation with one another and between each bystander third party that engaged in post-conflict affiliation and either opponent. As per Cordoni et al. (2023), we categorized the kinship as close (half- and full-siblings) and weak (first or second cousins and unrelated individuals).

To evaluate the occurrence of quadratic affiliative contacts we employed the standard PC-MC method used to demonstrate the presence of both reconciliation and

triadic contacts in social animals (de Waal and Yoshihara 1983; Arnold and Aureli 2007). After each conflict, we followed bystanders for a 3-min post-conflict period (PC). We employed a 3-min time window as it has been previously demonstrated that in pigs anxiety-related behaviors dropped within such a time window (Norscia, Collarini et al. 2021). For each PC, a corresponding 3-min matched control observation (MC) of the behavior of the same individuals was recorded. MC is usually carried out on a next possible day in the same social (i.e., at least four individuals present to provide the bystander with a similar opportunity to socially interact as in PC; Cordoni et al. 2023) and environmental context (same weather and time ± 1 h) on original bystanders, in absence of aggression in the previous 10 min. We distinguished three types of PC and MC observation pairs with respect to the timing of the first affiliative contact between bystanders: attracted pairs (AP, when the first affiliative contact occurred earlier in PC than MC or only in PC), dispersed pairs (DP, when the first affiliative contact occurred earlier in MC than PC or only in MC), or neutral pairs (NP, when the first affiliative contact occurred in both PC and MC at the same minute or in neither condition). As per de Waal and Yoshihara (1983), the occurrence of quadratic affiliative contacts can be confirmed if the number of attracted pairs is significantly higher than the number of dispersed pairs at the individual level. As for reconciliation and triadic contacts (Veenema et al. 1994), we calculated the individual Quadratic Contact Tendency (QCT%) as follows: $([AP - DP]/[AP + DP + NP]) \times 100$.

4.2.4 Statistical analyses

The statistical analyses (via SPSS 26.0) were carried out at the individual level and included pigs ($n = 49$) with at least three PC-MC pairs (which provides the opportunity to have at least one pair/type; Schino et al. 1998).

Owing to normal distribution of contact frequency data (Kolmogorov–Smirnov: $p_{males} = 0.109$; $p_{females} = 0.137$; $p_{Parma\ Black} = 0.200$; $p_{Large\ White} = 0.200$), we applied the parametric *t*-test for two independent samples to compare the bystander contact frequencies between males ($n = 28$) and females ($n = 21$) and between Parma Black ($n = 27$) and Large White ($n = 22$) breeds. We excluded Piedmont Black individuals because they did not comply with the three PC-MC pairs (minimum) condition.

In case of non-normal data distribution (Kolmogorov–Smirnov with Lilliefors correction: $p \leq 0.001$ for all samples), we also employed non-parametric statistics (Siegel and Castellan 1988). In particular, we applied the Wilcoxon's signed rank test for two dependent samples to compare attracted versus dispersed pairs and assess the presence of quadratic affiliation. Then, we assessed the factors possibly influencing such affiliation and the variation in the level of anxiety of the individuals involved in at least two affiliation contacts with other bystanders ($n = 41$), so as to ensure the possibility that bystander affiliation could occur in more than one condition. In particular, we applied the non-parametric Wilcoxon's signed rank test for two dependent samples to compare the frequency of affiliation 1) between close and weak kin bystanders; 2) between bystanders that were close or weak kin of either opponent. We used the Mann–Whitney test for two independent samples *via* Montecarlo

randomization to check for differences in the level anxiety behaviors between bystanders that initiated affiliation and those that received it. We applied the Friedman's test for $k > 2$ dependent samples to evaluate the potential influence of previous reconciliation contacts (QR), triadic contacts with the aggressee - unsolicited (QUV) and solicited (QSV) - triadic contacts with the aggressor - unsolicited (QUA) and solicited (QSA) - triadic contacts or no contact at all (QNP) on the probability of observing affiliation between bystanders. We used the same test to assess possible variations in anxiety levels in the absence of quadratic contact (AbsQ), after quadratic contact (PresQ) - both in the initiator and the receiver of the affiliation - and in MC. Because reconciliation, triadic contacts and bystander affiliation could occur at any time within the 3-min PC time window, the frequency of either bystander affiliation and anxiety behaviors were normalized by dividing it by the PC/MC minutes. We applied the Dunn post-hoc test for pairwise comparisons, with the significance level of probability (fixed at 0.05) adjusted downward using the Bonferroni correction.

4.3 Results

We found that the attracted pairs were significantly more than the dispersed pairs (Wilcoxon signed-ranks test: $n_{pigs} = 49$, $T = 310.00$, ties = 6, $p = 0.047$; **Figure 2**). Hence, the phenomenon of quadratic affiliative contacts was present in the pigs under study.

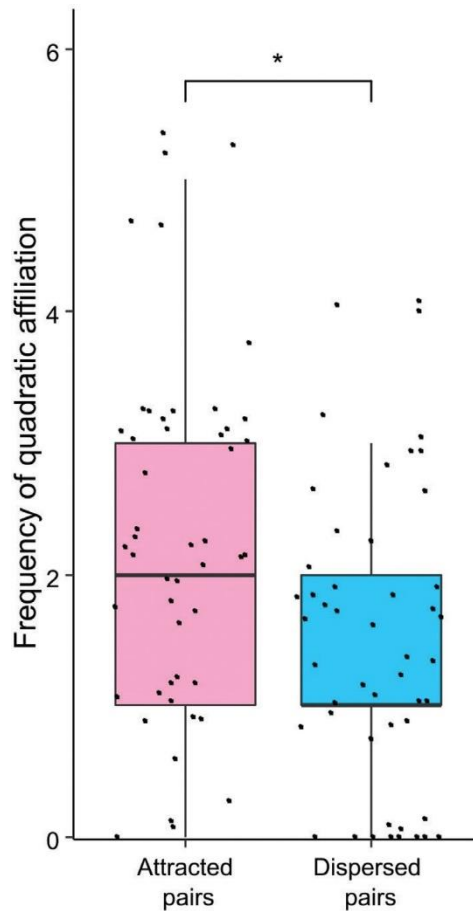


Figure 2. Differences between attracted and dispersed pairs (Wilcoxon signed-ranks test: $n_{\text{pigs}} = 49$, $T = 310.00$, ties = 6, $p = 0.047$). Horizontal lines: medians; box length: interquartile range; vertical line: minimum and maximum values in the data; points: data value distribution. NS = non significant; * = $p < 0.05$.

There was a significant variation in the frequency of anxiety-related behaviors across the three conditions (absence of quadratic affiliation, AbsQ; after quadratic affiliation, PresQ; MC; Friedman's test: $n_{\text{pigs}} = 41$, $\chi^2 = 48.082$, $df = 2$, $p < 0.001$; **Figure 3**). Specifically, we detected higher levels of anxiety behaviors in AbsQ than in PresQ (affiliation starting bystanders: $Q = 1.037$, $p < 0.001$; affiliation recipients: $Q = 1.073$, $p < 0.001$) and in AbsQ than in MC (affiliation starting bystanders: $Q = 1.012$, $p < 0.001$; affiliation recipients: $Q = 1.049$, $p < 0.001$), whereas there was no difference between PresQ and MC (affiliation starting bystanders: $Q = -0.024$, $p = 1.000$; affiliation recipients: $Q = -0.024$, $p = 1.000$). We found similar differences in the levels of anxiety behaviors in the bystanders that started the quadratic affiliation (Friedman test: $n_{\text{pigs}} = 41$, $\chi^2 = 48.515$, $df = 2$, $p < 0.001$) and in those that received the quadratic affiliation (Friedman test: $n_{\text{pigs}} = 41$, $\chi^2 = 52.583$, $df = 2$, $p < 0.001$). The level of anxiety-related behaviors did not differ between bystanders that initiated the quadratic affiliation and bystanders that received it (Mann–Whitney exact test: $U = 476.50$, $n_{\text{initiator}} = 32$, $n_{\text{receiver}} = 33$, $p = 0.301$).

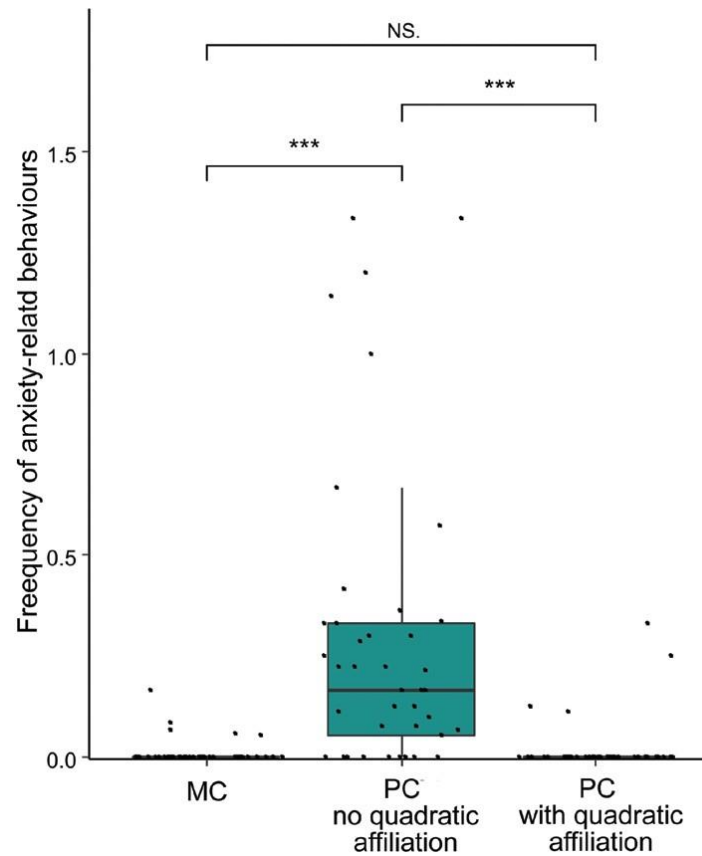


Figure 3. Differences in levels of anxiety-related behaviors across MC, absence of quadratic affiliation and after quadratic affiliation (Friedman's test: $n_{pigs} = 41$, $\chi^2 = 48.082$, $df = 2$, $p < 0.001$). Horizontal lines: medians; box length: interquartile range; vertical line: minimum and maximum values in the data; points: data value distribution. NS = non significant; *** = $P < 0.001$.

No difference in quadratic affiliation frequency was detected between males and females (t -test for independent samples: $n_{males} = 28$, $n_{females} = 21$, $df = 47$, $t = 1.121$, $p = 0.269$) and between breeds (t -test for independent samples; $n_{Parma\ Black} = 27$, $n_{Large\ White} = 22$, $df = 47$, $t = -1.103$, $p = 0.277$). Quadratic affiliation occurred more frequently between close- than weak-kin (Wilcoxon test $n_{pigs} = 41$, $t = 109.50$, ties = 10, $p = 0.006$; **Figure 4a**) and was significantly more frequent when the bystanders were closely related to either the aggressor (Wilcoxon signed-ranks test: $n_{pigs} = 41$, $t = 88.00$, ties = 3, $p < 0.001$; **Figure 4b**) or the aggressee (Wilcoxon signed-ranks test: $n_{pigs} = 41$, $t = 117.50$, ties = 2, $p < 0.001$; **Figure 4c**).

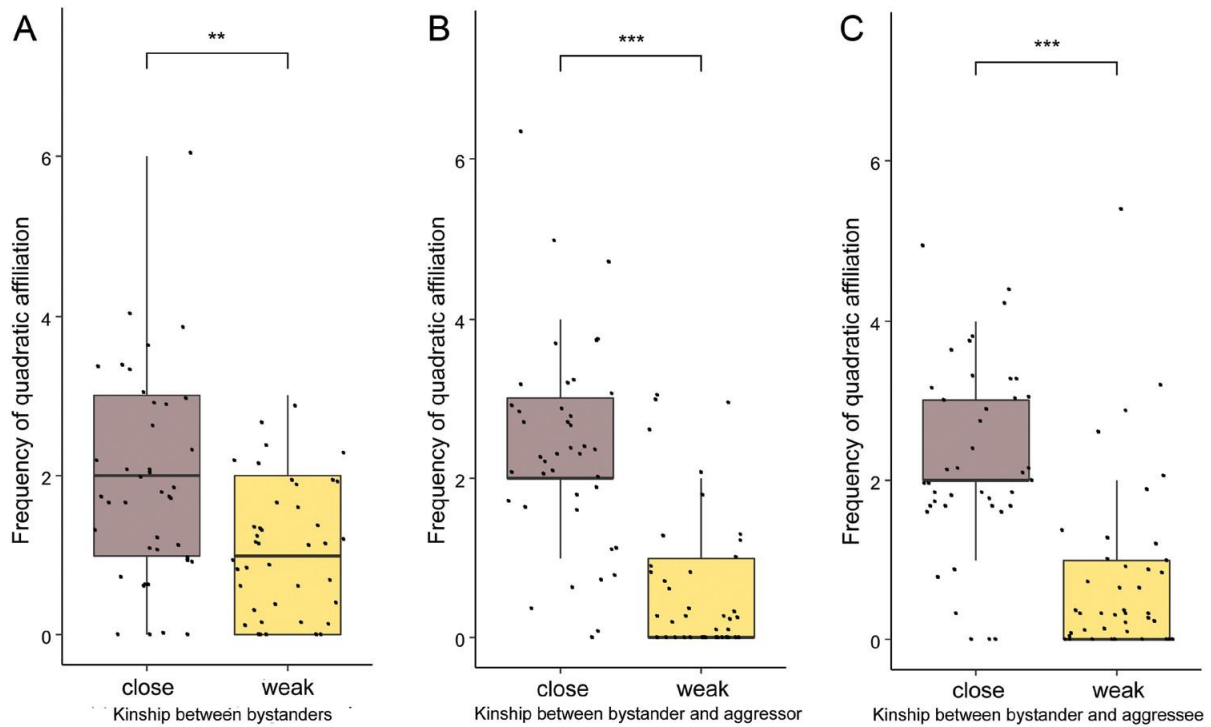


Figure 4. Box plot showing that: (A) quadratic affiliation occurred more frequently between close- than weak-kin (Wilcoxon test $n_{pigs} = 41$, $t = 109.50$, ties = 10, $p = 0.006$); (B) quadratic affiliation was significantly more frequent when the bystanders were closely related to the aggressor ((Wilcoxon signed-ranks test: $n_{pigs} = 41$, $t = 88.00$, ties = 3, $p < 0.001$); (C) quadratic affiliation was significantly more frequent when the bystanders were closely related to either the aggressee (Wilcoxon signed-ranks test: $n_{pigs} = 41$, $t = 117.50$, ties = 2, $p < 0.001$). Horizontal lines: medians; box length: interquartile range; vertical line: minimum and maximum values in the data; points: data value distribution. ** = $p < 0.01$, *** = $p < 0.001$.

We found that the likelihood of quadratic affiliation varied across conditions involving the previous presence of different types of post-conflict affiliation (reconciliation, QR; solicited/unsolicited contacts with the aggressee, QSV/QUV; solicited/unsolicited contacts with the aggressor, QSA/QUA) or no previous affiliation (QNP) (Friedman's test $n_{pigs} = 41$; $\chi^2 = 65.697$; $df = 5$; $p < 0.001$; **Figure 5**). In particular, the pairwise comparisons (via Bonferroni-Dunn post-hoc test) revealed highest levels of quadratic affiliation (**Figure 5**): 1) in absence of previous post-conflict affiliation than in presence of unsolicited contacts with the aggressee ($Q = -1.841$, $p < 0.001$); 2) in absence of previous post-conflict affiliation than in presence of unsolicited contacts with the aggressor ($Q = -1.329$, $p = 0.019$); 3) in absence of previous post-conflict affiliation than in presence of reconciliation ($Q = -1.780$, $p < 0.001$); 4) in presence of previous solicited contacts with the aggressee than in presence of unsolicited contacts with the aggressee ($Q = -2.024$, $p < 0.001$); 5) in presence of previous solicited contacts with the aggressee than in presence of unsolicited contacts with the aggressor ($Q = 1.512$, $p = 0.004$); and 6) in presence of previous solicited contacts with the aggressee than in presence of reconciliation ($Q = -1.693$, $p < 0.001$). No difference was found between other conditions (**Figure 5**). Hence, quadratic affiliation was least likely after reconciliation and unsolicited triadic

contact toward the aggressor and highest in absence of any previous post-conflict affiliation.

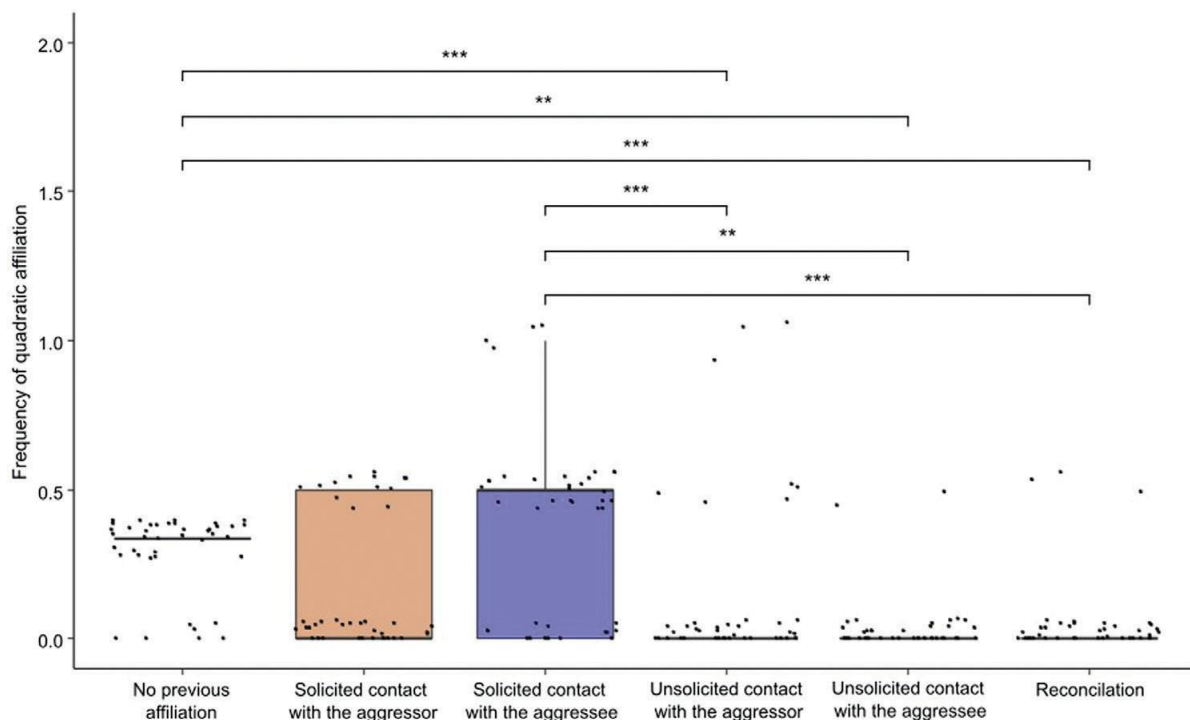


Figure 5. Differences in levels of quadratic affiliation across different conditions involving the previous presence of different types of post-conflict affiliation (reconciliation, QR; solicited/unsolicited contacts with the aggressor, QSV/QUV; solicited/unsolicited contacts with the aggressor, QSA/ QUA) or no previous affiliation (QNP): 1) QNP than in QUV ($Q = -1.841$, $p < 0.001$); 2) QNP than QUA ($Q = -1.329$, $p = 0.019$); 3) QNP than QR ($Q = -1.780$, $p < 0.001$); 4) QSV than QUV ($Q = -2.024$, $p < 0.001$); 5) QSV than QUA ($Q = 1.512$, $p = 0.004$); 6) QSV than QR ($Q = -1.693$, $p < 0.001$). No difference was found between other conditions (QUV vs. QR, $Q = 0.061$, $p = 1.000$; QUV vs. QUA, $Q = -0.512$, $p = 1.000$; QUV vs. QSA, $Q = -0.902$, $p = 0.434$; QR vs. QUA, $Q = -0.451$, $p = 1.000$; QR vs. QSA, $Q = -0.841$, $p = 0.626$; QUA vs. QSA, $Q = -0.390$, $p = 1.000$; QSA vs. QNP, $Q = -0.939$, $p = 0.346$; QSA vs. QSV, $Q = 1.122$, $p = 0.099$; QSV vs. QNP, $Q = 0.183$, $p = 1.000$). Horizontal lines: medians; box length: interquartile range; vertical line: minimum and maximum values in the data; points: data value distribution. NS = non-significant; ** = $p < 0.01$, *** = $p < 0.001$.

4.4 Discussion

Our study shows that quadratic affiliation is present in the domestic pig because individuals not directly involved in aggression as opponents or third party (hereafter bystanders) were significantly more likely to affiliate with one another after witnessing a conflict (PC) than in absence of conflict (MC) (*Prediction 1 supported*; **Figure 2**). This result indicates that also in pigs - as it occurs in other species - aggressive events can affect the interactions of the entire social group (De Marco et al. 2010; Schino and Sciarretta 2015). As pointed out in the introduction, the domestic pig qualifies as a species in which quadratic affiliation can be adaptive to restore homeostasis as aggression is used to establish or change dominance dynamics that are relevant to the whole group (Meikle et al. 2010; D'Eath 2002; Andersen et al. 2004; Norring et al.

2019). Moreover, in pigs aggression can affect stress physiology and anxiety behaviors - with the latter especially occurring in individuals that are not the opponents - and social affiliation can work in decreasing anxiety (Fernandez et al. 1994; Norscia, Collarini et al. 2021).

We found that quadratic affiliation reduced post-conflict anxiety in the bystanders down to baseline levels (*Prediction 2a supported*; **Figure 3**). This finding is consistent with the fact that in pigs affiliation (not just quadratic) can accelerate post-conflict anxiety decrease (Norscia, Collarini et al. 2021) and that anxiety in the aggressee can be reduced after a spontaneous affiliation initiated by a third party (Cordoni et al. 2023). This result supports the finding that quadratic affiliation leads to post-conflict reduction of anxiety-related behaviors in the bystanders, as observed in Japanese macaques (Daniel and Alves 2015), hamadryas baboons (Judge and Mullen 2005) and one group (out of two) of Tonkean macaques (De Marco et al. 2010). Depending on how it is expressed, quadratic affiliation might require that a bystander appreciates the emotional behavior of another bystander toward a shared referent (in our case the conflict) and enacts subsequent regulatory mechanisms (elements of social appraisal, as per Walle et al. 2017). Such behavioral mechanisms may reduce the divergence between the bystander actual internal state and its prediction of the other bystander's emotional state and possibly change its own experience (intrinsic regulation) or the experience of the contacted subject (extrinsic regulation) (Zaki and Williams 2013; Prochazkova and Kret 2017; Walle et al. 2017; Cordoni et al. 2023). Our results show that the bystanders that initiated the quadratic contact showed comparable levels of anxiety behaviors as the bystanders that received such contact, which was followed by a decrease of anxiety behaviors in both affiliation initiators and recipients (*Prediction 2b not supported*). An explanation for this result is that the anxiety reduction experienced by the affiliation recipient may be a byproduct of the quadratic contact enacted by the initiator to reduce its own anxiety (intrinsic regulation; *sensu* Zaki and Williams 2013). However, we cannot exclude that elements of extrinsic regulation (Zaki and Williams 2013) underlie quadratic contacts, which also change the emotional state of others (i.e., anxiety reduction in the bystander receiving the affiliation). Consistently, it has been observed in pigs that the unsolicited triadic affiliation that a third party engage with the aggressee can work in reducing the anxiety in the aggressee and not in the third party (Cordoni et al. 2023). Of course, more of a parsimonious explanation may be that quadratic affiliation in pigs is a mere automatic reaction to a stressor (the conflict). However, in the pigs under study, quadratic affiliation was not distributed randomly across group members because it was influenced by kinship (**Figure 4**) and previous post-conflict affiliation (**Figure 5**), which may point toward some elements of social appraisal, required also when pigs engage in different types of non-random post-conflict triadic affiliation (Cordoni et al. 2023).

Indeed, we found that quadratic affiliation was affected by kinship because affiliation occurred most frequently between close kin (**Figure 4a**) and when either opponent was close kin of bystanders (**Figure 4b, c**). Kinship with the opponents appeared to be one possible driver of quadratic affiliation in hamadryas baboons, even

though non-kin of both opponents could engage in such affiliation at comparable rates (Judge and Mullen 2005). Social proximity between bystanders - more than kinship - enhanced their quadratic affiliation in monkeys (hamadryas baboons, Judge and Mullen, 2005; Tonkean macaque, De Marco et al. 2010). This finding can be explained in the light of the social organization of feral pigs and wild boars. Under wild conditions, adult males tend to disperse but sows form social groups of variable size that are based on close kinship (for both males and females) and, in general, the dispersal patterns are reflected in the kin-based social organization (Stolba and Wood-Gush 1989; d'Eath and Turner 2009; Podgórski, Lusseau et al. 2014; Podgórski, Scandura et al. 2014). Hence, social closeness can be associated with close kinship in pigs, at least in the wild. There is evidence, although limited so far, that pigs can recognize kin after separation (McLeman et al. 2005). The involvement of close kin as opponent in the conflict or as partner in quadratic affiliation probably makes such affiliation more relevant to the inclusive fitness of the bystander, as it has also been observed that triadic contacts in the same pig population occurred especially between close kin (Cordoni et al. 2023).

Another non-random aspect of pig quadratic affiliation is that in our sample such affiliation was less likely to occur after reconciliation or unsolicited triadic affiliation toward the aggressee whereas it was most likely in absence of any previous post-conflict affiliation or after solicited triadic contacts toward the aggressee (**Figure 5**). Contacts initiated by a bystander toward the aggressor did not change the probability of observing quadratic affiliation (**Figure 5**). These results suggest that quadratic affiliation may come into play as a substitute for other post-conflict behaviors. It is possible that when the conflict is resolved directly by the opponents, via peacemaking, group homeostasis is restored and quadratic affiliation is not required. Reconciliation can work in reducing the anxiety (measured via self-directed behaviors) especially in the aggressee (e.g., domestic goats, *Capra hircus*: Schino 1998; macaques, *Macaca* spp: Aureli et al. 1989; Kutsukake and Castle 2001) but also in the aggressor (e.g., hamadryas baboons: Romero et al. 2009) even though in tolerant species anxiety reduction may not be an issue (e.g., crested macaques *Macaca nigra*: Duboscq et al. 2014). Importantly, in hamadryas baboons - where the phenomenon of quadratic affiliation is present (Judge and Mullen 2005) - witnessing reconciliation can reduce anxiety in the bystanders (Judge and Bachmann 2013). In the same pig population, Cordoni et al. (2023) found that only unsolicited triadic contacts toward the aggressee reduced anxiety in the recipient of the affiliation. Hence, also in this case the adaptive value of quadratic affiliation may be reduced, which may explain why quadratic contacts plummeted when third parties spontaneously affiliated with the aggressee. On the other hand, Cordoni et al. (2023) also found that contacts initiated by a bystander toward the aggressee did not decrease their anxiety whereas solicited contacts with the aggressor after a conflict did not differ from chance (hence such contacts were not elicited by the aggression per se). These previous findings (Cordoni et al. 2023) may explain why quadratic affiliation was not affected by these

types of post-conflict behaviors and probably remained necessary to restore group homeostasis.

Finally, sex and breed did not influence the likelihood of such affiliation (*Predictions 3a and 3b supported*), despite the fact that different breeds can diverge in the level of aggressiveness (and related implications), and that males and females show different social organisation as adults, with males usually dispersing and females forming kin-bonded groups (Breuer et al. 2003; Podgórski, Lusseau et al. 2014; Podgórski, Scandura et al. 2014). However, the pigs of our study group were not pure breed and males were castrated. Thus, possible inter-breed or inter-sex differences - if present - may have been reduced under the detection level. Consistently, a previous study on the same population found no sex or breed dependent differences in the anxiety-related behaviors expressed after conflict (Norscia, Collarini et al. 2021).

In conclusion, this study showed for the first time that quadratic affiliation is present in domestic pigs and that - as it occurs for triadic contacts (Cordoni et al. 2023) - it is not randomly distributed across individuals. The main limit of this study is that males were castrated - possibly affecting testosterone levels (Raeside et al. 1997) - and individuals were mixed breed which may have reduced the effect of some individual factors on quadratic affiliation. Hormonal correlates would also be useful to assess stress levels in relation to conflict and subsequent affiliation. Future studies could focus on these aspects but while breed differences may be assessed in multibreed extensive farming, it may prove difficult to reliably collect biological samples for hormonal essays before and after different types of post-conflict affiliation without perturbing the animals and to find multiple reproductive males coexisting in association with female groups. Despite these constraints, we can hypothesize that quadratic affiliation in pigs might not just be a mere response automatically generated by the aggression. Quadratic affiliation led to the reduction of anxiety behaviors in both the interacting bystanders (affiliation initiator and recipient) which suggests that such affiliation is more self- than other-oriented. The fact that quadratic affiliation was not randomly distributed across bystanders but it was modulated by kinship and previous post-conflict behaviors (which may or may not have restored group homeostasis) suggests - as an important evolutionary convergence with primates - that further cognitive processes may be in place, such as elements of social appraisal that can enhance the benefits that a subject obtains by engaging in the affiliation (i.e., own anxiety reduction).

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Ethical Statement: The study - purely observational with no manipulation whatsoever - did not require ethical approval. This study was conducted in accordance with the Italian law Dlg. 4/2014 n. 26 (based on European provisions), which regulates the use of animals in experimental and scientific research. This study is part of the broader project “So.Pig” (Department of Life Sciences and Systems Biology, University of Turin) and PhD PON project “GreenPig” co-funded by the Ministry of University and Research (MUR). These projects focus on domestic pigs that are not raised in a natural environment, with the aim of better understanding social dynamics and their relationship with welfare, the effect of domestication on behavior, best management practices, and possible evolutionary divergences and convergences with other social species, including humans. The subjects under study were reared on an ethical farm (Parva Domus, Turin, Italy) and were housed in a 13 ha enclosure that were parts of a natural woodland/grassland habitat. The pigs were able to move freely and avoid each other if wanted. The study did not require the pigs to be removed from their group either temporarily or on a longer-term basis. Periodically, a vet visited the pigs for vaccination or health problems. We took all possible precautions to avoid any fear, distress or harm on the pigs and to reduce the impact of our presence on their environment. We did not interact with the pigs, which were free to perform their usual maintenance and social activities.

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4.5 References

- Andersen, I. L., Nævdal, E., Bakken, M., & Bøe, K. E. (2004). Aggression and group size in domesticated pigs, *Sus scrofa*: 'When the winner takes it all and the loser is standing small.' *Animal Behaviour*, 68(4), 965–975. <https://doi.org/10.1016/j.anbehav.2003.12.016>
- Arnold, K., & Aureli, F. (2007). Postconflict reconciliation. In *Primates in Perspective* (2nd ed., pp. 608–625).
- Aureli, F. (1997). Post-conflict anxiety in nonhuman primates: The mediating role of emotion in conflict resolution. *Aggressive Behavior*, 23(5), 315–328. [https://doi.org/10.1002/\(sici\)1098-2337\(1997\)23:5<315::aid-ab2>3.0.co;2-h](https://doi.org/10.1002/(sici)1098-2337(1997)23:5<315::aid-ab2>3.0.co;2-h)
- Aureli, F., Cords, M., & van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: A predictive framework. *Animal Behaviour*, 64(3), 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Aureli, F., Van Schaik, C. P., & Hooff, V. (1989). Functional aspects of reconciliation among captive long tailed macaques (*Macaca fascicularis*). *American Journal of Primatology*, 19(1), 39–51.
- Breuer, K., Sutcliffe, M. E. M., Mercer, J. T., Rance, K. A., Beattie, V. E., Sneddon, I. A., & Edwards, S. A. (2003). The effect of breed on the development of adverse social behaviours in pigs. *Applied Animal Behaviour Science*, 84(1), 59–74. [http://dx.doi.org/10.1016/S0168-1591\(03\)00147-3](http://dx.doi.org/10.1016/S0168-1591(03)00147-3)
- Camerlink, I., Coulange, E., Farish, M., Baxter, E. M., & Turner, S. P. (2018). Facial expression as a potential measure of both intent and emotion. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-35905-3>
- Camerlink, I., & Turner, S. P. (2013). The pig's nose and its role in dominance relationships and harmful behaviour. *Applied Animal Behaviour Science*, 145(3–4), 84–91. <https://doi.org/10.1016/j.applanim.2013.02.008>
- Camerlink, I., Turner, S. P., Ursinus, W. W., Reimert, I., & Bolhuis, J. E. (2014). Aggression and affiliation during social conflict in pigs. *PLoS One*, 9(11), e113502. <https://doi.org/10.1371/journal.pone.0113502>
- Caratti, S., Rossi, L., Sona, B., Origlia, S., Viara, S., Martano, G., Torre, C., & Robino, C. (2010). Analysis of 11 tetrameric STRs in wild boars for forensic purposes. *Forensic Science International: Genetics*, 4(5), 339–342. <https://doi.org/10.1016/j.fsigen.2010.07.001>
- Chapman, C. A., & Valenta, K. (2015). Costs and benefits of group living are neither simple nor linear. *Proceedings of the National Academy of Sciences*, 112(48), 14751–14752. <https://doi.org/10.1073/pnas.1519760112>
- Cheney, D. L., & Seyfarth, R. M. (1989). Redirected aggression and reconciliation among vervet monkeys *Cercopithecus aethiops*. *Behaviour*, 110, 258–275. <http://dx.doi.org/10.1163/156853989X00501>
- Cordoni, G., Comin, M., Collarini, E., Robino, C., Chierto, E., & Norscia, I. (2022). Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: Cognitive and functional implications. *Animal Cognition*. <https://doi.org/10.1007/s10071-022-01688-4>

- Cordoni, G., & Norscia, I. (2014). Peace-making in marsupials: The first study in the red-necked wallaby (*Macropus rufogriseus*). *PLoS One*, 9(1), e86859. <https://doi.org/10.1371/journal.pone.0086859>
- Cordoni, G., & Palagi, E. (2015). Being a victim or an aggressor: Different functions of triadic post-conflict interactions in wolves (*Canis lupus lupus*). *Aggressive Behavior*, 41(6), 526–536. <https://doi.org/10.1002/ab.21590>
- Cords, M., & Thurnheer, S. (2010). Reconciling with valuable partners by long-tailed macaques. *Ethology*, 93(4), 315–325. <https://doi.org/10.1111/j.1439-0310.1993.tb01212.x>
- D'Eath, R. B. (2002). Individual aggressiveness measured in a resident-intruder test predicts the persistence of aggressive behaviour and weight gain of young pigs after mixing. *Applied Animal Behaviour Science*, 77(4), 267–283. [https://doi.org/10.1016/s0168-1591\(02\)00077-1](https://doi.org/10.1016/s0168-1591(02)00077-1)
- D'Eath, R. B., & Turner, S. P. (2008). The natural behaviour of the pig. In *The Welfare of Pigs* (pp. 13–45). Springer Netherlands.
- Daniel, J. R., & Alves, R. L. (2015). Postconflict affiliation among bystanders in a captive group of Japanese macaques (*Macaca fuscata*). *International Journal of Primatology*, 36(2), 259–268. <https://doi.org/10.1007/s10764-015-9822-8>
- Das, M. (2000). Conflict management. *Natural Conflict Resolution*. University of California Press.
- De Marco, M., Cozzolino, A., Dessì-Fulgheri, R., & Thierry, F. (2010). Conflicts induce affiliative interactions among bystanders in a tolerant species of macaque (*Macaca tonkeana*). *Animal Behaviour*, 80(2), 197–203.
- De Waal, F. B. M. (2000). Primates - a natural heritage of conflict resolution. *Science*, 289(5479), 586–590. <https://doi.org/10.1126/science.289.5479.586>
- De Waal, F. B. M., & Aureli, F. (1996). Consolation, reconciliation and a possible cognitive difference between macaques and chimpanzees. In T. Bard & S. Parker (Eds.), *Reaching into thought: The minds of great apes* (pp. 80–110). Cambridge University Press.
- De Waal, F. B. M., & Aureli, F. (1997). Conflict resolution and distress alleviation in monkeys and apes. In C. S. Carter, I. I. Lederhendler, & B. Kirkpatrick (Eds.), *The integrative neurobiology of affiliation* (pp. 317–328). New York Academy of Sciences.
- De Waal, F. B. M., & Preston, S. D. (2017). Mammalian empathy: behavioural manifestations and neural basis. *Nature Reviews Neuroscience*, 18(8), 498–509. <https://doi.org/10.1038/nrn.2017.72>
- De Waal, F. B. M., & van Roosmalen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioural Ecology and Sociobiology*, 5(1), 55–66. <https://doi.org/10.1007/bf00302695>
- De Waal, F. B. M., & Yoshihara, D. (1983). Reconciliation and redirected affection in rhesus monkeys. *Behaviour*, 85(3–4), 224–241. <https://doi.org/10.1163/156853983x000237>
- Duboscq, J., Agil, M., Engelhardt, A., & Thierry, B. (2014). The function of postconflict interactions: new prospects from the study of a tolerant species of primate. *Animal Behaviour*, 87, 107–120. <http://dx.doi.org/10.1016/j.anbehav.2013.10.018>

- Fernandez, X., Meunier-Salaün, M. C., & Mormede, P. (1994). Agonistic behavior, plasma stress hormones, and metabolites in response to dyadic encounters in domestic pigs: interrelationships and effect of dominance status. *Physiology & Behavior*, 56(5), 841–847. [https://doi.org/10.1016/0031-9384\(94\)90313-1](https://doi.org/10.1016/0031-9384(94)90313-1)
- Fraser, O. N., & Bugnyar, T. (2010). Do ravens show consolation? Responses to distressed others. *PLoS One*, 5(5), e10605. <https://doi.org/10.1371/journal.pone.0010605>
- Fraser, O. N., Koski, S. E., Wittig, R. M., & Aureli, F. (2009). Why are bystanders friendly to recipients of aggression? *Communicative & Integrative Biology*, 2(3), 285–291. <https://doi.org/10.4161/cib.2.3.8718>
- Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. *Proceedings of the National Academy of Sciences USA*, 105(25), 8557–8562. <https://doi.org/10.1073/pnas.0804141105>
- Goumon, S., & Špinka, M. (2016). Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Animal Cognition*, 19(3), 501–511. <https://doi.org/10.1007/s10071-015-0950-5>
- Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2(2), 186. <https://doi.org/10.12966/abc.05.08.2014>
- Jensen, P. (1986). Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 16(2), 131–142. [https://doi.org/10.1016/0168-1591\(86\)90105-x](https://doi.org/10.1016/0168-1591(86)90105-x)
- Jensen, P. (2002). Behaviour of pigs. In P. Jensen (Ed.), *The ethology of domestic animals: An introductory text* (3rd ed., pp. 159–172).
- Judge, P. G. (1991). Dyadic and triadic reconciliation in pigtail macaques *Macaca nemestrina*. *American Journal of Primatology*, 23, 225–237. <https://doi.org/10.1002/ajp.v23:4>
- Judge, P. G., & Bachmann, K. A. (2013). Witnessing reconciliation reduces arousal of bystanders in a baboon group (*Papio hamadryas hamadryas*). *Animal Behaviour*, 85(5), 881–889. <https://doi.org/10.1016/j.anbehav.2013.02.011>
- Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, 69(6), 1345–1355. <https://doi.org/10.1016/j.anbehav.2004.08.016>
- Kling, D., Tillmar, A. O., & Egeland, T. (2014). Familias 3 – Extensions and new functionality. *Forensic Science International: Genetics*, 13, 121–127. <https://doi.org/10.1016/j.fsigen.2014.07.004>
- Kutsukake, N., & Castles, D. L. (2004). Reconciliation and post-conflict third-party affiliation among wild chimpanzees in the Mahale Mountains, Tanzania. *Primates*, 45, 157–165. <https://doi.org/10.1007/s10329-004-0082-z>
- Leone, A., Mignini, M., Mancini, G., & Palagi, E. (2010). Aggression does not increase friendly contacts among bystanders in geladas (*Theropithecus gelada*). *Primates*, 51, 299–305. <https://doi.org/10.1007/s10329-010-0204-8>

- Løvendahl, P., Damgaard, L. H., Nielsen, B. L., Thodberg, K., Su, G., & Rydhmer, L. (2005). Aggressive behaviour of sows at mixing and maternal behaviour are heritable and genetically correlated traits. *Livestock Production Science*, 93(1), 73–85. <https://doi.org/10.1016/j.livprodsci.2004.11.008>
- Majolo, B., de Bortoli Vizioli, A., & Schino, G. (2008). Costs and benefits of group living in primates: Group size effects on behaviour and demography. *Animal Behaviour*, 76(4), 1235–1247. <https://doi.org/10.1016/j.anbehav.2008.06.008>
- Marino, L., & Colvin, C. M. (2015). Thinking pigs: A comparative review of cognition, emotion, and personality in *Sus domesticus*. *International Journal of Comparative Psychology*, 28. <https://doi.org/10.46867/ijcp.2015.28.00.04>
- McFarland, R., & Majolo, B. (2011). Reconciliation and the costs of aggression in wild Barbary macaques (*Macaca sylvanus*): A test of the integrated hypothesis. *Ethology*, 117(10), 928–937. <https://doi.org/10.1111/j.1439-0310.2011.01948.x>
- McFarland, R., & Majolo, B. (2012). The occurrence and benefits of postconflict bystander affiliation in wild Barbary macaques (*Macaca sylvanus*). *Animal Behaviour*, 84(3), 583–591. <https://doi.org/10.1016/j.anbehav.2012.06.010>
- McHugh, M. L. (2012). Interrater reliability: The kappa statistic. *Biochemia Medica*, 22(3), 276–282. <https://doi.org/10.11613/bm.2012.031>
- McLeman, M. A., Mendl, M., Jones, R. B., White, R., & Wathes, C. M. (2005). Discrimination of conspecifics by juvenile domestic pigs, *Sus scrofa*. *Animal Behaviour*, 70(2), 451–461. <https://doi.org/10.1016/j.anbehav.2004.11.013>
- Meikle, D. B., Drickamer, L. C., Vessey, S. H., Arthur, R. D., & Rosenthal, T. L. (2010). Dominance rank and parental investment in swine (*Sus scrofa domesticus*). *Ethology*, 102(8), 969–978. <https://doi.org/10.1111/j.1439-0310.1996.tb01174.x>
- Norring, M., Valros, A., Bergman, P., Marchant-Forde, J. N., & Heinonen, M. (2019). Body condition, live weight and success in agonistic encounters in mixed parity groups of sows during gestation. *Animal*, 13(2), 392–398. <https://doi.org/10.1017/s1751731118001453>
- Norscia, I., Coco, E., Robino, C., Chierito, E., & Cordoni, G. (2021). Yawn contagion in domestic pigs (*Sus scrofa*). *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-020-80545-1>
- Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>
- Palagi, E., & Cordoni, G. (2009). Postconflict third-party affiliation in *Canis lupus*: Do wolves share similarities with the great apes? *Animal Behaviour*, 78(4), 979–986. <https://doi.org/10.1016/j.anbehav.2009.07.017>
- Palagi, E., Dall'Olio, S., Demuru, E., & Stanyon, R. (2014). Exploring the evolutionary foundations of empathy: Consolation in monkeys. *Evolution and Human Behavior*, 35(4), 341–349. <https://doi.org/10.1016/j.evolhumbehav.2014.04.002>
- Palagi, E., & Norscia, I. (2013). Bonobos protect and console friends and kin. *PLOS ONE*, 8(11), e79290. <https://doi.org/10.1371/journal.pone.0079290>

- Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLOS ONE*, 9(6), e99875. <https://doi.org/10.1371/journal.pone.0099875>
- Podgórski, T., Scandura, M., & Jędrzejewska, B. (2014). Next of kin next door: Philopatry and socio-genetic population structure in wild boar. *Journal of Zoology*, 294(3), 190–197.
- Prochazkova, E., & Kret, M. E. (2017). Connecting minds and sharing emotions through mimicry: A neurocognitive model of emotional contagion. *Neuroscience and Biobehavioral Reviews*, 80, 99–114. <https://doi.org/10.1016/j.neubiorev.2017.05.013>
- Puga-Gonzalez, I., Butovskaya, M., Thierry, B., & Hemelrijk, C. K. (2014). Empathy versus parsimony in understanding post-conflict affiliation in monkeys: Model and empirical data. *PLOS ONE*, 9(3), e91262. <https://doi.org/10.1371/journal.pone.0091262>
- Raeside, J. I., Friendship, R. M., & Vrablic, O. E. (1997). Effects of castration on early postnatal development of male accessory sex glands in the domestic pig. *European Journal of Endocrinology*, 137, 287–292. <https://doi.org/10.1530/eje.0.1370287>
- Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109, 42–50. <https://doi.org/10.1016/j.physbeh.2012.11.002>
- Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: Emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, 18(2), 517–532. <https://doi.org/10.1007/s10071-014-0820-6>
- Romero, T., Castellanos, M. A., & De Waal, F. B. M. (2011). Post-conflict affiliation by chimpanzees with aggressors: Other-oriented versus selfish political strategy. *PLOS ONE*, 6(7), e22173. <https://doi.org/10.1371/journal.pone.0022173>
- Romero, T., Colmenares, F., & Aureli, F. (2009). Testing the function of reconciliation and third-party affiliation for aggressors in hamadryas baboons (*Papio hamadryas hamadryas*). *American Journal of Primatology*, 71(1), 60–69. <https://doi.org/10.1002/ajp.20619>
- Schino, G. (1998). Reconciliation in domestic goats. *Behaviour*, 135(3), 343–356. <http://dx.doi.org/10.1163/156853998793066302>
- Schino, G., Aureli, F., & Rosati, L. (1998). Intragroup variation in conciliatory tendencies in captive Japanese macaques. *Behaviour*, 135(7), 897–912. <https://doi.org/10.1163/156853998792640314>
- Schino, G., & Sciarretta, M. (2015). Effects of aggression on interactions between uninvolved bystanders in mandrills. *Animal Behaviour*, 100, 16–21. <https://doi.org/10.1016/j.anbehav.2014.11.013>
- Siegel, S., & Castellan, N. J., Jr. (1988). *Nonparametric statistics for the behavioral sciences*.
- Silk, J. B. (2006). Practicing Hamilton's rule: Kin selection in primate groups. In *Cooperation in Primates and Humans* (pp. 25–46). Springer Berlin Heidelberg.
- Špinka, M. (2017). Behaviour of pigs. In *The ethology of domestic animals: An introductory text* (pp. 214–227). CABI.

- Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science*, 48(2), 419–425. <https://doi.org/10.1017/s0003356100040411>
- Turner, S. P., D'Eath, R. B., Roehe, R., & Lawrence, A. B. (2010). Selection against aggressiveness in pigs at re-grouping: Practical application and implications for long-term behavioural patterns. *Animal Welfare*, 19(S1), 123–132. <https://doi.org/10.1017/S0962728600002323>
- Walle, E. A., Reschke, P. J., Camras, L. A., & Campos, J. J. (2017). Infant differential behavioral responding to discrete emotions. *Emotion*, 17(7), 1078–1091. <https://doi.org/10.1037/emo0000307>
- Veenema, H. C., Das, M., & Aureli, F. (1994). Methodological improvements for the study of reconciliation. *Behavioural Processes*, 31, 29–38. [http://dx.doi.org/10.1016/0376-6357\(94\)90035-3](http://dx.doi.org/10.1016/0376-6357(94)90035-3)
- Yamamoto, C., Ishibashi, T., Kashiwagi, N., & Amano, N. (2020). Functions of post-conflict bystander affiliations toward aggressors and victims in bottlenose dolphins. *Scientific Reports*, 10, 3776. <http://dx.doi.org/10.1038/s41598-020-60423-6>
- Yamamoto, C., Morisaka, T., Furuta, K., Ishibashi, T., Yoshida, A., et al. (2015). Post-conflict affiliation as conflict management in captive bottlenose dolphins (*Tursiops truncatus*). *Scientific Reports*, 5, 14275. <http://dx.doi.org/10.1038/srep14275>
- Zaki, J., & Williams, W. C. (2013). Interpersonal emotion regulation. *Emotion*, 13(5), 803–810. <https://doi.org/10.1037/a0033839>

5. Nose-clip use in semi-free ranging pigs reduces rooting without disrupting affiliative behaviour or causing prolonged stress

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Abstract

Domestic pigs (*Sus scrofa*) raised under natural conditions can show their complete behavioural repertoire. However, rooting behaviour can have a great impact on the environment. In the context of the promotion of farm animal welfare and environmental concerns, this study investigated the potential of nose-clips as a less invasive alternative to nose-rings for the management of rooting behaviour of free-ranging pigs. We collected behavioural data and salivary cortisol levels on two groups: an experimental group (EG; n = 17) with nose-clips and a control group (CG; n = 17) without nose-clips. After the nose-clipping, we observed a temporary increase in anxiety-related behaviour and cortisol levels during the first week after nose-clipping, followed by a return to pre-application levels in the following weeks. We found a temporary decrease in affiliative interactions involving the nose during the first week after the application of nose-clips, whereas no differences in affiliative interactions without nose contact and aggression levels were observed. Moreover, nose-clips effectively reduced destructive excavation behaviours without leading to a simultaneous increase in other exploratory behaviours. In conclusion, our findings show that nose-clips could be a solution that mitigates destructive rooting while preserving social interactions and animal welfare. Further research is essential to consolidate these findings and assess the long-term implications of this approach.

5.1 Introduction

In recent years, many efforts have been made to increase physical, social, and emotional welfare of farm animals. Although with some variations, the classical standards of animal welfare are based on the so-called five freedoms, including: (1) freedom from hunger and thirst; (2) freedom from discomfort; (3) freedom from pain, injury, and disease; (4) freedom to express natural behaviour; and (5) freedom from fear and distress (e.g., McCausland, 2014). Over the years, it has been realized that ensuring the minimum requirements is not enough (Miller and Chinnaduraiand, 2023). In this respect, the focus has been on enabling the animals to freely interact with their environment and effectively manage challenging situations (Dantzer, 2002; Puppe et al., 2007). From an animal welfare perspective, it is crucial to provide animals with the opportunity not only to minimize negative experiences but also to enhance positive ones (Edgar, 2013).

Negative experiences can elevate individual anxiety, detrimentally impacting animal welfare. Anxiety is an affective state characterised by tension and/or agitation, often expressed via self-directed behaviour (e.g. scratching; Troisi, 2002). Anxiety can be connected to stress, as it often manifests behaviourally alongside a physiological stress response (Barros and Tomaz, 2002; Troisi, 2002; Bourin et al., 2007). Indeed, anxiety-related behaviours appear often linked to the cortisol-mediated stress response, the intensity and duration of which depends on the stressor (Beerda et al., 2000, Thompson, 2011; Kubota et al., 2014; Rinaldi, 2019). Acute stress - depending on the occurrence of specific eliciting stressors - occurs over minutes or hours, whereas chronic stress - depending on long-term aversive conditions - occurs on a time scale from weeks to years (Epel et al., 2018). Several studies have noted that stress can elevate both aggression and subsequent anxiety-related behaviours in both human and non-human animals (Rodgers et al., 1997; Ferrari et al., 1998; Sandi and Haller, 2015). Conversely, positive social interactions, including affiliative behaviours, play a crucial role in promoting and enhancing animal health and welfare status (Rault, 2012, Mellor, 2015a, Rault 2019).

Domestic pigs are animals with complex socio-emotional interactions (Marino and Colvin 2015). In pigs, exploration, rooting and foraging are highly motivated behaviours and their performance is intrinsically rewarding (Stolba and Wood-Gush, 1989, Boissy et al., 2007). Preventing pigs from engaging in these behaviours is associated with a number of negative experiences such as frustration, boredom, anger and stress (Panksepp, 2005, Mellor, 2015b, Kells 2022). Instead, providing opportunities for pigs to perform this kind of behaviour can alleviate some negative experiences and thus improve their welfare (Kells 2022). Moreover, an adequate social environment can improve the welfare of pigs (Goumon et al., 2020). Social interactions are considered positive social behaviour in the domestic pig (Blackshaw, A.M. Hagelsø 1990; Camerlink et al., 2016) and it has been observed that affiliative behaviours represent an effective mechanism to buffer anxiety after stressful events (Norscia et al., 2021b).

When domestic pigs are raised in free or semi-free ranging conditions, they often exhibit social behavioural patterns akin to their wild counterparts, such as wild boars (Jensen, 1986; Stolba and Wood-Gush, 1989). Pigs can i) exhibit anxiety-related behaviours including scratching, head/body rubbing, yawning, and vacuum chewing, particularly following stressful events (Norscia et al., 2021b), ii) show emotional contagion, and iii) freely choose their affiliative partners, with affiliation serving as an anxiety reducer (Norscia et al., 2021a; Norscia et al., 2021b).

While extensive farming practices can be beneficial for pig welfare, raising pigs in a natural environment poses challenges for farmers. Pigs may spend a significant proportion (about 52%) of their activity budget engaging in rooting and foraging behaviours (Stolba and Wood-Gush, 1989). The motivation to root increases not only when pigs are searching for food, but also when they are exposed to new areas (Horrell, 1992; Studnitz and Jensen, 2002; Studnitz et al., 2007). However, the impact of pig rooting activity on the paddock is considerable and it leads to substantial depletion of the vegetation cover (Edge et al., 2005). For this reason, the use of nose-rings has been employed to reduce the environmental impact of rooting (Horrell et al., 2000, 2001). Nose-rings can cause discomfort when the nose contacts a hard surface and therefore promote less impactful exploratory behaviours, such as sniffing and grazing (Studnitz et al., 2003 a,b). Nevertheless, the practice of nose-ringing has raised many ethical concerns with suggestions that it causes distress and negative consequences for animal welfare (Farm Animal Welfare Council, 1996), which would go against the above mentioned five freedom standards. To address this issue, Farm Animal Welfare Council (1996) recommends assessing the motivational state underlying the need for rooting, and addressing the frustration that arises from preventing rooting.

Thus, raising pigs in natural habitats while avoiding environmental depletion poses a 'farming conundrum,' as natural habitats promotes the freedom to express natural behaviour but the resulting environmental impact may induce farmers to opt in favour of indoor facilities, thus ultimately reducing this freedom. The solution to this puzzling issue is yet to be determined. In this study, we aim to explore if pig sociality, anxiety-related behaviours, and invasive exploratory activities are affected by the application of nose-clips—smaller and supposedly less invasive than nose-rings. To this purpose, we compared a physiological biomarker of stress (salivary cortisol), anxiety-related behaviours (frequency of displacement activities), excavation/exploratory activity, affiliative contacts, and aggression recorded in both clipped (experimental group) and non-clipped (control group) pigs over a four-week period before and after nose-clip application onto the subjects belonging to the experimental group. Our predictions are detailed below.

5.1.1 Prediction 1

In pigs, the occurrence of anxiety-related behaviours rise in response to stressful events or disturbance (Norscia et al., 2021b). From a physiological

standpoint, an increase in salivary cortisol levels is observed as a consequence of arousal, including arousal-related distress in the absence of other arousal-inducing factors (Koopmans et al., 2005). If the practice of nose-clipping - as it occurs for nose-ringing (Studnitz et al., 2003a, b)- impacts the physical and psychological well-being of pigs, we anticipate that cortisol levels would be higher after than before nose-clip application (*Prediction 1a*). Additionally, we anticipate that the variation of anxiety-related behaviours would follow the change in cortisol levels (*Prediction 1b*).

5.1.2 Prediction 2

One of the possible outcomes of anxiety increase – besides behavioural inhibition related to threat – is an increase of aggression (Rodgers et al., 1997; Ferrari et al., 1998; Porges. 2003; Sandi and Haller, 2015). Although the relationship between anxiety and aggression may not always be straightforward, anxiety can lead to aggression increase in several mammalian species, including rats (Neuman et al., 2010), monkeys (especially reactive aggression; Paschek et al., 2019) and domestic dogs (Kleszcz et al., 2022).

Given this perspective, we anticipate that the levels of aggression may be higher after than before nose-clip application (*Prediction 2a*). Moreover, in their natural environment, domestic pigs frequently engage in social behaviours involving the nose, such as nose-to-body and nose-to-nose contacts and these interactions allow animals to recognize each other and maintain social hierarchies (Stokey and Gonyou, 1998; Kristensen et al., 2001; Mendl et al., 2002; McLeman et al., 2004; Horback, 2014). The use of nose-rings can reduce nose contacts, causing discomfort to pigs (Horrell et al., 2000, 2001). If the same applies to nose-clips, we expect that affiliative behaviours, especially those involving the nose, would decrease following the application of the nasal clips in the experimental group (*Prediction 2b*).

5.1.3 Prediction 3

Nose-ring application can lead to a reduction of excavation, via rooting and rasping, and promote less invasive exploratory behaviours such as sniffing and foraging (Horrell et al., 2000, 2001; Studnitz et al., 2003 a,b). If the same applies to nose-clip application on free-ranging pigs, we expect that the frequencies of rooting and rasping would be lower after than before nose-clip application (*Prediction 3a*). Concurrently, we expect that the frequencies of exploratory behaviours, such as grazing/browsing and foraging, would be higher after than before nose-clip application (*Prediction 3b*).

5.2 Materials and Methods

5.2.1 Study group and site

Data collection was conducted in July-August 2021 on a group of semi-free ranging domestic pigs (*Sus scrofa*) at the Ethical Farm “Parva Domus” located at Cavagnolo (Turin, Italy). The animals could move freely and forage in a natural woodland habitat, within a fenced area of 3 ha. The study group was composed by 34 early adult pigs (Parma Black breed) including 16 males (9-10 months) and 18 females (8-9 months). The males had been castrated via the removal of testes within their first days of life, whereas females were potentially reproductive but the reproductive male was kept in a separate enclosure. The pigs were provided with feed (Ciclo Unico P, SILDAMIN®) once daily between 8:30 and 10:30 and water was available *ad libitum*. The subjects could supplement their feed intake with roots, leaves, fruits, seeds and bark naturally available in the environment.

5.2.2 Nose-clip application and study design

We applied a 2x3 design, considering two groups and three periods. To this purpose we divided the study subjects into two groups of the same size (17 subjects, 8 males and 9 females) - Experimental Group (EG, in which subjects had nose-clips applied) and Control Group (CG). In CG, the individuals received a nose stitch – same nose handling as EG individuals – but the nose-clips were not actually applied. The individuals were randomly assigned to either group while ensuring a similar sex balance. Moreover, we considered three observational periods over a four-week span: the week (7 days) before nose-clip application (hereafter, PRE) and the first (7 days) and third (7 days) weeks after nose-clip application (hereafter, **POST1** and **POST2** for the first and third weeks, respectively). (see **Figure 1**).

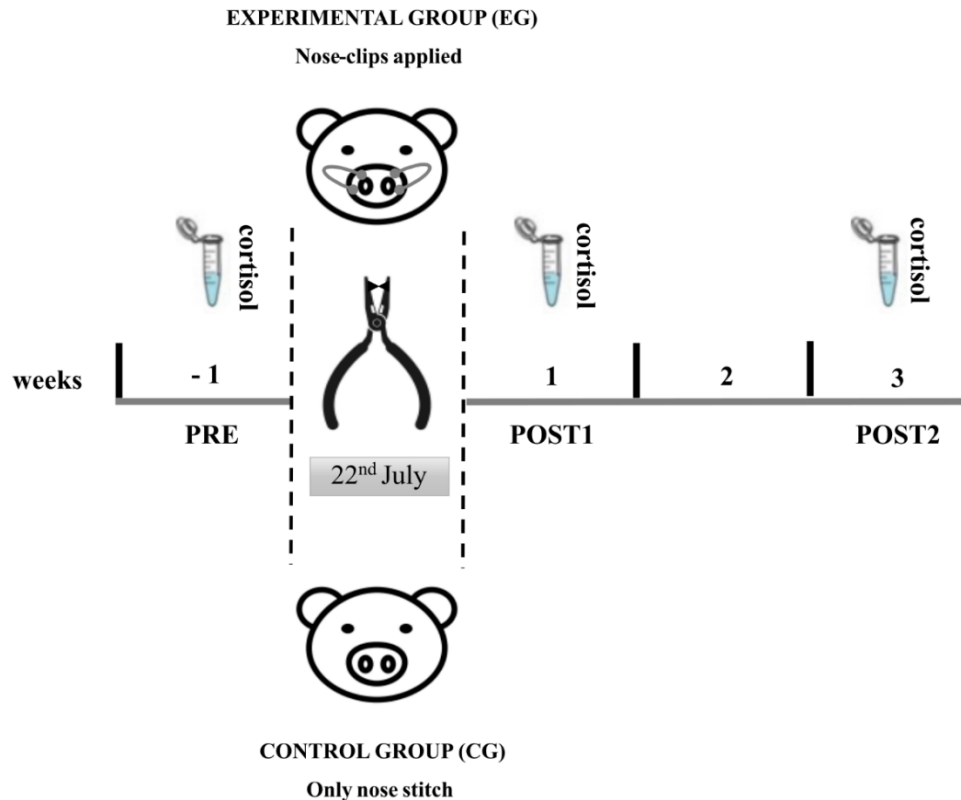


Figure 1. Graphic abstract showing the protocol employed in the current study. A period of a week (-1) before the application of the nose-clips (PRE) and a period of three weeks (1, 2, 3) after the application of the nose-clips (POST) are represented with a dark grey line divided in equal parts (weeks) by black vertical lines. On the 22th of July 2021, the nose-clips were applied with pliers to the individuals of the experimental group (EG; upper part of the figure) whereas in the control group (CG; lower part of the figure) the pigs' noses were pinched with the pliers, as if a clip were to be applied, but without actually applying the clip.

Nose-clips (Veteur ©, four/pigs) were applied with pliers to the subjects of the EG after a week from the start of fieldwork (on the 22nd of July 2021). Nose-clips consisted of open steel wire rings (25 mm in diameter), clipped to the rim of the nose (each pig was fitted with 4 nose-clips to minimise the risk of the animals losing the clips and to avoid a second application; see **Figure 2**). Concurrently, the subjects of the CG received the same procedure of the EG except that no clips were applied. The entire procedure (covering subjects of both EG and CG) lasted around 1.5 hr. Nose-clip application was not specifically carried out for this study: it was performed by the farmer as part of the management to allow pigs to roam around a natural setting without causing damage to the habitat, especially via rooting and digging. However, for the purpose of this study part of the pigs were not clipped (control group) until the end of the data collection period.

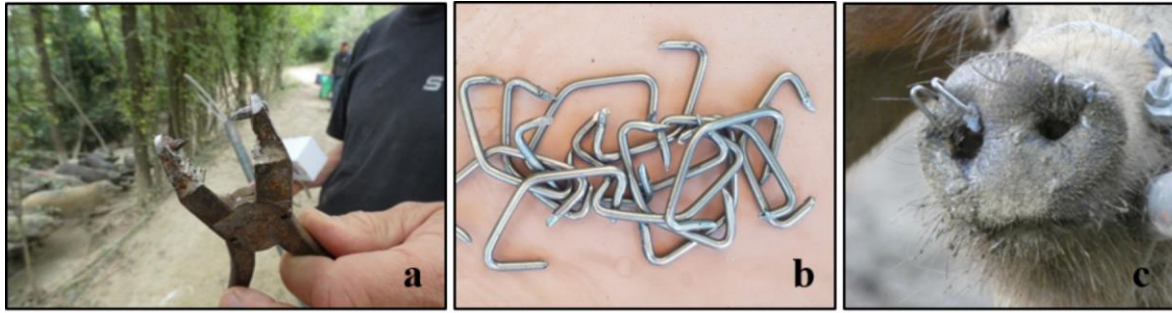


Figure 2. Photos representing (a) the pliers used for applying nose-clips to pigs, (b) the model of nose-clips applied to animals (Veteur © model N. 5; INSVET, S.A., Huesca Spain), and (c) an individual of the experimental group with four nose-clips applied (2 x upper part of each nostril).

5.2.3 Eco-ethological data collection

Environmental data and pig size estimation. Field data collection was carried out from 15th July to 13th August. Maximum and minimum values of temperature (°C) and humidity (%) were collected daily during the entire data collection period, via a portable Meteo Station (Elegant; model EOX-9901). From the maximum and minimum temperature and humidity values we calculated mean daily temperature and humidity. Two sets of size measurements - including body length and chest girth (cm) - were taken on each pig one day before the start and one day after the end of the data collection period. Body length and chest girth can explain around 90% of the weight variation and can therefore provide a reliable estimate of pig live weight (Mutua et al., 2011). The measurements were taken by the experimenters with the aid of a clothing tape while the subjects were feeding (with no necessity to constrain them). Body length was measured from the neck base to the tail base right above the shoulder; chest girth was determined by measuring the circumference of the chest area behind the forelegs (Birteeb et al., 2015). Considering pig age (8-10 months), weight (kg) was estimated by applying the following formula (Mutua et al., 2011): $0.39 \times \text{length} + 0.64 \times \text{girth}$.

Behavioural data. Anxiety-related (vacuum-chewing, scratching/body-rubbing, head/body-shaking, and yawning), affiliative, aggressive, excavation (rooting and rasping), and exploratory (grasping/browsing and foraging) behaviours were collected via focal animal sampling (Altmann, 1974), which consists of recording all the behaviours performed by an individual in a given time window (in our case 15-min focal audio-videos in the time window from 07:30 am to 08:30 pm). One/two focal samplings were conducted daily for each individual throughout the data collection period, at different times of the day, to obtain 3 hr of observation/individual/phase (a total of 9 hr; PRE: 3hr; POST1: 3hr; POST2: 3hr), following a rotation of the time schedule, in order to obtain a balanced observation effort (PRE/POST1/POST2). The behaviours were categorized following the ethogram used by Norscia et al., (2021b) and described in **Table 1**. As regards affiliation, we considered i) body contact interactions without the use of the nose (e.g., contact sitting/resting, head over), and ii) body contact interactions with the use of the nose (e.g., nose-to-nose, nose-to-body). The videos

were recorded by Maria Traversa with a Panasonic HC-V180 camera and the camera was hand-held to follow the focal pig. The videos were then analysed via freeware VLC 3.0.6 and extension Jump-to-Time. Before starting systematic video-analysis Ivan Norscia and Giada Cordini supervised Maria Traversa in a training period of 30 hours to reach an interobserver reliability score (Cohen's k) never below 0.80.

Table 1. Affiliative, aggressive, anxiety-related, excavation and exploratory behaviours of domestic pig considered in this study

Behavioural patterns	Description
Affiliative behaviours NOT involving the nose	
Rest/Sit in contact	Two pigs lay down or sit touching one another with their bodies
Head-over	A pig puts its head above the neck of a companion
Affiliative behaviours involving the nose	
Nose-body contact	A pig touches/pushes with its nose a body part of a companion (excluding nose)
Nose-nose contact	A pig touches with its nose the nose of a companion
Aggressive behaviours	
Aggressive bite	A pig opens its mouth and close its teeth tight on a companion's small piece of flesh, including tail
Aggressive head-nocking	A pig lurches or jerks its head hitting a companion
Aggressive lifting	A pig attempts to displace a companion by lifting or levering it with snout or head
Aggressive mount	A pig forces a companion to move away by rising upon its rear
Aggressive push	A pig presses its head, neck, shoulder or body against a companion thus causing its moving
Avoidance	A pig changes its route or moves away while a companion is approaching it
Chase	A pig pursues a companion
Flee	A pig runs away while it is chasing by a companion
Anxiety-related behaviours	
Head/Body shaking	A pig vigorously shakes its head and/or body
Scratching/body-rubbing	A pig uses its legs or a substrate to rub part of its body
Vacuum-chewing	A pig chews with empty mouth
Yawning	A pig performs deep, long inhalation with open mouth
Excavation behaviours	
Rasping	A pig rasps the ground on the surface, in searching for food

Rooting	A pig digs the ground with its snout and eats from roots and other underground material
Exploratory behaviours	
Foraging	A pig sniffs, touches, scrapes, nibbles digs things and explores around the floor or roots floor or other things above the floor level
Grazing/ Browsing	A pig feeds from grass and plant leaves, bark

To determine dominance relationships, each day during feeding all the aggressive events occurring over a time window of 15-min were noted (*all-occurrences animal sampling*; Altmann, 1974). Although distributed over a span of several meters to reduce aggression, the food was still provided in a limited area, which caused multiple agonistic events between pigs. For each aggression, the winner and loser of agonistic interactions were identified, with the loser being the opponent that retreated from the aggression or fled. We collected a total of 705 and 700 aggressive events in PRE and POST2 periods, respectively.

5.2.4 Saliva sample collection and hormonal analyses

Saliva samples were taken from a subgroup of individuals (17 subjects, 7 from EG, 10 from CG) because the individuals were roaming within a fenced area of 3ha and it was not possible to collect all samples from all individuals. For each individual in this subgroup, three saliva samples were taken at different times: a first saliva sample was collected in the week prior to nose-clip application (PRE), a second saliva sample was collected at the end of the first week after nose-clip application (POST1), a third saliva sample was collected at the end of the third week after nose-clip application (POST2). Each saliva collection round was carried out in the same morning slot, from 7.30 to 8.30 am, before the pigs were fed. Samples were collected by the experimenters wiping the surface of the pig's oral cavity with cotton swabs (Murase et al., 2019). Saliva samples were placed in a freezer at -20°C within one hour from collection and kept in dry ice when moved to the labs of the Department of Molecular Biotechnology and Health Sciences Department (University of Torino) where they were kept in a -20°C freezer until the hormonal assays were carried out.

Chemicals. Analytical standards (purity > 98%) cortisol and internal standard (IS) cortisol-d3 were purchased from Sigma and LCG (Milan, Italy) respectively. Stock solutions for both analytes and IS were prepared at a concentration of 1 µg/mL for analytes and 25 µg/mL for IS using methanol and stored at -20°C until use. Further dilutions were obtained in formic acid 0.1% in water/methanol 80:20. HPLC-MS grade ethyl ether, HPLC-MS hyper grade acetonitrile and methanol, and formic acid were purchased from VWR International (Milan, Italy). Ultrapure water was obtained from a VWR Water System.

Sample preparation. Swabs were fortified with cortisol-d3 internal standard (IS) (30 ng/mL final concentration) and 20 mL of ethyl ether were added to the samples.

Organic solvent was recovered after shaking and ultrasonication for 5 minutes. The extracts were dried under a gentle stream of nitrogen and the dried samples were reconstituted with 500 μ L of 0.1% formic acid in water: MeOH 80:20 solution.

Liquid Chromatography-Mass Spectrometry. The chromatographic separations were run on a Supelco Ascentis C18 column, 100 $\times$ 2.1 mm, 3 μ m particle size (Merck-Sigma Aldrich, Milan, Italy) with a Isolute Env+ 30 $\times$ 2.1 mm (Biotage, Milan, Italy) as a pre analytical cartridge, both thermostated at 40°C. Injection volume was 20 μ L and flow rate 300 μ L/min both for loading (C, formic acid 0.1%) and eluent (A, formic acid 0.1%; B, 0.1% formic acid in acetonitrile) solutions. Gradient mobile phase composition was adopted: 70:30 to 0:100 A/B in 12 min. A Shimadzu Nexera (Shimadzu, Kyoto, Japan) X2 UPLC SIL 30AC coupled with a Sciex 5500 Q-trap mass spectrometer (Sciex, Framingham, MA, USA) equipped with a Turbo Ion Spray atmospheric pressure interface (ESI ion source) was used. The LC column effluent was delivered into the ion source using nitrogen as sheath and auxiliary gas. The ion source temperature was set at 300°C and the needle voltage at the 4.5 kV value. The acquisition method used was previously optimized for the analytes ions and spectra were acquired in the positive ion MRM mode. Two transitions were selected for Cortisol, three for IS and all the MRM parameters for transition (Q1 $\rightarrow$ Q3), Declustering Potential, Entrance Potential, Collision energy and Collision Cell Exit Potential are reported in **Table 2**. The analytical protocol follows the protocol described and validated by Hauser et al., (2008).

Table 2. Table shows the parameters set to carry out the chromatographic assays of domestic pig saliva samples, for declustering potential, input potential, collision energy and collision cell output potential.

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
Cortisol	363.0	121.0	120	7	30	15
	363.0	309.0	120	7	21	25
Internal Standard	366.0	121.0	59	11	29	18
	366.0	330.0	59	11	21	22
	366.0	272.0	59	11	27	16

Abbreviations: DP = declustering potential; EP = input potential; CE = collision energy; CXP = collision cell output potential

5.2.5 Statistical analyses

Via the R 'steepness' package (<https://CRAN.R-project.org/package=steepness>; Leiva and de Vries, 2022), we calculated the dominance rank of the pigs, expressed as Normalized David's scores (**NDSS**). NDSS were assessed for each subject from decided aggressive encounters (i.e., it was possible to clearly distinguish a winner and a loser). The individual frequencies of decided encounters were entered in a sociomatrix. NDSS were determined from a

dyadic dominance index (D_{ij}) in which the observed proportion of wins (P_{ij}) is corrected for the chance occurrence of the observed outcome. This chance occurrence is calculated based on a binomial distribution with each subject having an equal chance of winning or losing in every aggressive encounter (de Vries, Stevens and Vervaecke, 2006). We determined the NDS-based hierarchy in the two periods (PRE and POST2) by ordering the individuals according to their NDS based ranks.

As a first step we ran a series of preliminary analyses to check that periods (PRE/POST) and groups (EG/CG) were comparable. As a second step, we verified whether the nose-clip application modified the estimated weight gain and ranking positions in the study subject. Data were normally distributed and so a t-test for two independent samples was applied to: i) compare the mean temperature (Kolmogorov-Smirnov test: $n_{\text{days_PRE}} = 7$, $n_{\text{days_POST}} = 21$; $z \geq 0.113$, $p \geq 0.111$) and the mean humidity between PRE and POST the application of nose-clips (Kolmogorov-Smirnov test: $n_{\text{days_PRE}} = 7$, $n_{\text{days_POST}} = 21$; $z \geq 0.129$, $p \geq 0.200$); data are not paired as there is no specific association between a specific day in the period PRE and a specific day in the period post; ii) compare the individual NDS difference ($\text{NDS}_{\text{POST}} - \text{NDS}_{\text{PRE}}$) between EG and CG (Kolmogorov-Smirnov test: $n_{\text{subjects}} = 17$, $z \geq 0.358$, $p \geq 0.877$); iii) to compare the individual estimated weight gain (estimated $\text{weight}_{\text{POST}} - \text{estimated weight}_{\text{PRE}}$) between the EG and CG (Kolmogorov-Smirnov test: $n_{\text{subjects}} = 17$, $z \geq 0.713$, $p \geq 0.653$);

Then, we assessed whether the fluctuation of anxiety-related behaviours before and after nose-clip application followed a similar variation to the cortisol levels informing stress levels. To this purpose, we compared anxiety-related behaviour frequencies and salivary cortisol levels across three periods: the week before the nose-clip application (PRE), the first week after the nose-clip application (POST1) and the third week after the nose-clip application (POST2). Due to the small sample size ($n_{\text{EG}} = 7$; $n_{\text{CG}} = 10$) we applied the non-parametric Friedman test for k-dependent samples to compare the levels of salivary cortisol across periods. Furthermore, we compared the frequencies of anxiety-related behaviours displayed by individuals in both groups across the same three periods (PRE, POST1 and POST2). Owing to the prevalence of non-normal data distribution (Lilliefors-corrected Kolmogorov-Smirnov test: $z \geq 0.202$, $0.001 \leq p \leq 0.063$), we applied the Friedman test for k-dependent samples to compare the frequencies of anxiety-related behaviours across periods.

Finally, to evaluate possible effects of nose-clips on excavation/exploratory activity and social behaviours we compared the frequencies of anxiety-related, affiliative, aggressive, excavation (foraging + rasping) and exploratory (grasping/browsing + foraging) behaviours (see **Table 1**) across three periods (PRE: during the week before the application of nose-clips; POST1: during the first week after the application of nose-clips; POST2: during the third week after the application of nose-clips).

In the case of non-normal data distributions (Lilliefors-corrected Kolmogorov-Smirnov test: $0.001 \leq p \leq 0.200$), we applied non-parametric statistics for analysis (Siegel and Castellan 1988). In particular, we applied the non-parametric Friedman

test for k-dependent samples to compare the hourly frequencies of excavation behaviour (EG, CG), exploratory behaviour (EG, CG), affiliative behaviours involving the nose (EG, CG), proximity (EG, CG) and aggression (CG). Exact probabilities were selected for small sample sizes, following Mundry and Fischer (1998). Due to a normal distribution (Lilliefors-corrected Kolmogorov-Smirnov test: $p \geq 0.05$), we applied the parametric One-Way Anova test for k-dependent samples, to compare the hourly frequency of affiliative behaviours not involving the nose (EG, CG) and aggression (EG). The statistical significance was set at 0.05.

5.3 Results

5.3.1 Preliminary analyses: cross period and cross group comparability

Temperature and humidity estimate. The periods preceding (PRE) and following (POST) the application of nose-clips ($n_{\text{days_PRE}} = 7$, $n_{\text{days_POST}} = 21$) had comparable mean temperature ($\text{Mean}_{\text{PRE}} \pm \text{SE}$: $24.81^\circ\text{C} \pm 0.65$; $\text{Mean}_{\text{POST}} \pm \text{SE}$: $23.37^\circ\text{C} \pm 0.51$; t-test for two independent samples: $t = 1.094$, $df = 26$, $p = 0.103$) and humidity ($\text{Mean}_{\text{PRE}} \pm \text{SE}$: $63.00\% \pm 2.81$; $\text{Mean}_{\text{POST}} \pm \text{SE}$: $68.88\% \pm 1.91$; t-test for two independent samples: $t = -0.442$, $df = 26$, $p = 0.109$).

Estimated ranking position and weight difference. During PRE, the subjects of the experimental (EG) and control (CG) groups ($n_{\text{subjects_EG}} = 17$, $n_{\text{subjects_CG}} = 17$) had similar ranking positions expressed via NDS ($\text{Mean}_{\text{EG}} \pm \text{SE}$: 15.763 ± 0.953 ; $\text{Mean}_{\text{CG}} \pm \text{SE}$: 17.236 ± 0.588 ; t-test for two independent samples: $t = 0.316$, $df = 32$, $p = 0.198$) and size ($\text{Mean}_{\text{EG}} \pm \text{SE}$: $109.581\text{kg} \pm 2.551$; $\text{Mean}_{\text{CG}} \pm \text{SE}$: $109.501\text{kg} \pm 3.651$; t-test for two independent samples: $t = -0.018$, $df = 32$, $p = 0.986$). Hence, groups and periods were comparable. During the POST2 the subjects of EG and CG ($n_{\text{subjects_EG}} = 17$, $n_{\text{subjects_CG}} = 17$) showed similar NDS based rank differences ($\text{Mean}_{\text{EG}} \pm \text{SE}$: -0.103 ± 0.353 ; $\text{Mean}_{\text{CG}} \pm \text{SE}$: 0.103 ± 0.180 ; t-test for two independent samples: $t = 0.514$, $df = 32$, $p = 0.611$) and size differences ($\text{Mean}_{\text{EG}} \pm \text{SE}$: $6.576\text{kg} \pm 21.353$; $\text{Mean}_{\text{CG}} \pm \text{SE}$: $5.897\text{kg} \pm 1.754$; t-test for two independent samples: $t = -0.308$, $df = 32$, $p = 0.760$). Therefore, the nose-clip influenced neither the overall weight gain nor the dominance rank of the experimental subjects.

5.3.2 Prediction 1

Cortisol and anxiety behaviours levels. We found a significant difference in both frequencies of anxiety-related behaviours (Friedman test: $n_{\text{EG}} = 17$, $\chi^2 = 12.125$, $df = 2$, $p = 0.002$; **Figure 3a**) and salivary cortisol levels (Friedman test: $n_{\text{EG}} = 7$, $\chi^2 = 8.380$, $df = 2$, $p = 0.002$, **Figure 3b**) in EG across the three periods. Regarding anxiety-related behaviours, we detected an increase from PRE to POST1 (Dunn test, PRE vs. POST1: $Q = -1.118$, $p = 0.003$) and a decrease from POST1 to POST2 (Dunn test, POST2 vs. POST1: $Q = 0.824$, $p = 0.049$), whereas there was no difference between the PRE and POST2 periods (Dunn test, PRE vs. POST2: $Q = -0.249$, $p = 1.000$). Similarly, regarding salivary cortisol levels we detected an increase from PRE to

POST1 (Dunn test, PRE vs. POST1: $Q = -1.286$, $p = 0.048$) and a decrease from POST1 to POST2 (Dunn test, POST2 vs. POST1: $Q = -1.286$, $p = 0.048$), whereas there were no significant differences between PRE and POST2 (Dunn test, PRE vs. POST2: $Q = -0.000$, $p = 1.000$). In contrast, we found no significant differences in both frequencies of anxiety-related behaviour (Friedman test: $n_{CG} = 17$, $\chi^2 = 1.524$, $df = 2$, $p = 0.467$) and salivary cortisol levels (Friedman test: $n_{CG} = 10$, $\chi^2 = 0.359$, $df = 2$, $p = 0.091$) of CG across the three periods (PRE, POST1, POST2).

These results show that both anxiety and stress levels increased just after the nose-clip application (POST 1) but then decreased to pre-application levels (POST 2).

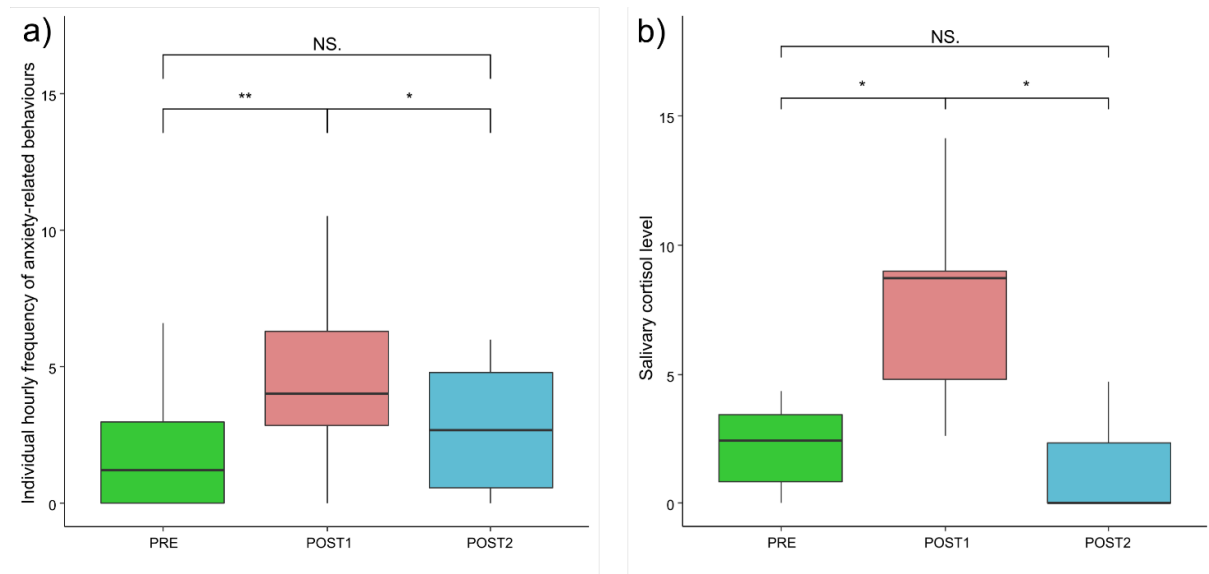


Figure 3. Box plots showing that in experimental group (EG) of free-ranging domestic pigs the levels of anxiety-related behaviours (a) and salivary cortisol (b) were higher in the first week after nose-clipping (POST1) than in the week before (PRE) and the third week after nose-clipping (POST2), there were no differences between the week before (PRE) and the third week after nose-clipping (POST2). Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS=non-significant, **= $p < 0.01$, *= $p < 0.05$.

5.3.4 Prediction 2

Social behaviours

Body contacts with nose. In EG the levels of affiliative behaviours involving the nose (i.e. nose-to-nose and nose-to-body contacts) were significantly lower in POST1 than in PRE and POST2, whereas they did not differ between PRE and POST2 (Friedman test: $n_{EG} = 17$, $\chi^2 = 10.698$, $df = 2$, $p = 0.005$; Dunn test, PRE vs POST1: $Q = 2.915$, $p = 0.011$; PRE vs POST2: $Q = 0.668$, $p = 1.000$; POST1 vs POST2: $Q = -2.487$, $p = 0.039$, **Figure 4a**). In CG, the frequencies of body contacts involving the nose did not significantly differ across the three periods (Friedman test: $n_{CG} = 17$, $\chi^2 = 2.375$, $df = 2$, $p = 0.305$). Hence, the application of nose-clips temporarily reduced the levels of affiliative contacts involving the nose, which were restored to pre-application levels within the third week.

Body contacts without nose. The frequencies of body contacts not involving the nose did not show any significant difference across the three periods (PRE, POST1, POST2) in both EG and CG (One-way ANOVA test: $n_{EG} = 17$, $\chi^2 = 0.077$, $df = 2$, $p = 0.926$; $n_{CG} = 17$, $\chi^2 = 2.636$, $df = 2$, $p = 0.082$, **Figure 4b**). Thus, nose-clips did not reduce affiliation not involving the nose.

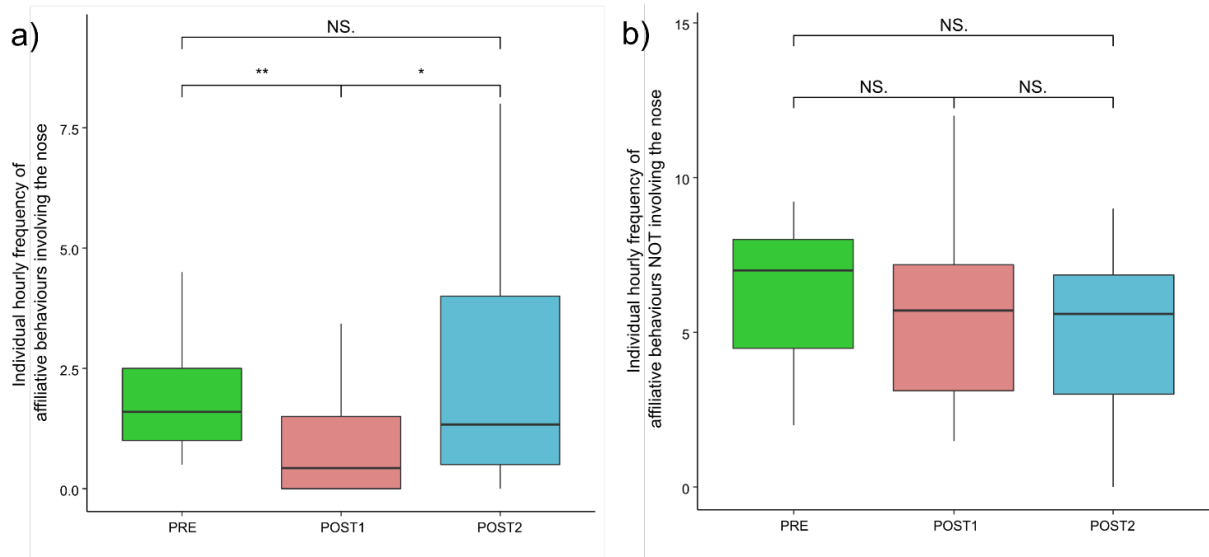


Figure 4. Box plots showing that: (a) in experimental group of free-ranging domestic pigs (EG) the levels of affiliative behaviours involving the nose were lower in the first week after nose-clipping (POST1) than in the week before (PRE) and the third week after nose-clipping (POST2), whereas there were no differences between the week before (PRE) and the third week after nose-clipping (POST2); (b) in experimental group of free-ranging domestic pigs (EG) there were no differences in the levels of affiliative behaviours not involving the nose. Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS=non-significant, ** = $p < 0.01$, * = $p < 0.05$.

Aggression. Frequencies of aggression did not significantly differ across the three periods in both EG and CG (One-way ANOVA test, $n_{EG} = 17$, $\chi^2 = 2.922$, $df = 2$, $p = 0.063$; Friedman test, $n_{CG} = 17$, $\chi^2 = 4.388$, $df = 2$, $p = 0.111$). Hence, the application of nose-clips did not cause an increase of aggressive events.

5.3.5 Prediction 3

Excavation and exploratory behaviours

In EG, frequencies of excavation behaviour (rooting plus rasping) were significantly lower in both POST1 and POST2 compared to PRE, whereas there were no differences between POST1 and POST2 (Friedman test: $n_{EG} = 17$, $\chi^2 = 13.935$, $df = 2$, $p = 0.001$; Dunn test, PRE vs POST1: $Q = 3.087$, $p = 0.006$; PRE vs POST2: $Q = 3.087$, $p = 0.006$; POST1 vs POST2: $Q = 0.000$, $p = 1.000$; **Figure 5a**). Moreover, exploratory activity levels (taking/navigation plus foraging) did not differ across the three periods (Friedman test, $n_{EG} = 17$, $\chi^2 = 0.567$, $df = 2$, $p = 0.753$, **Figure 5b**).

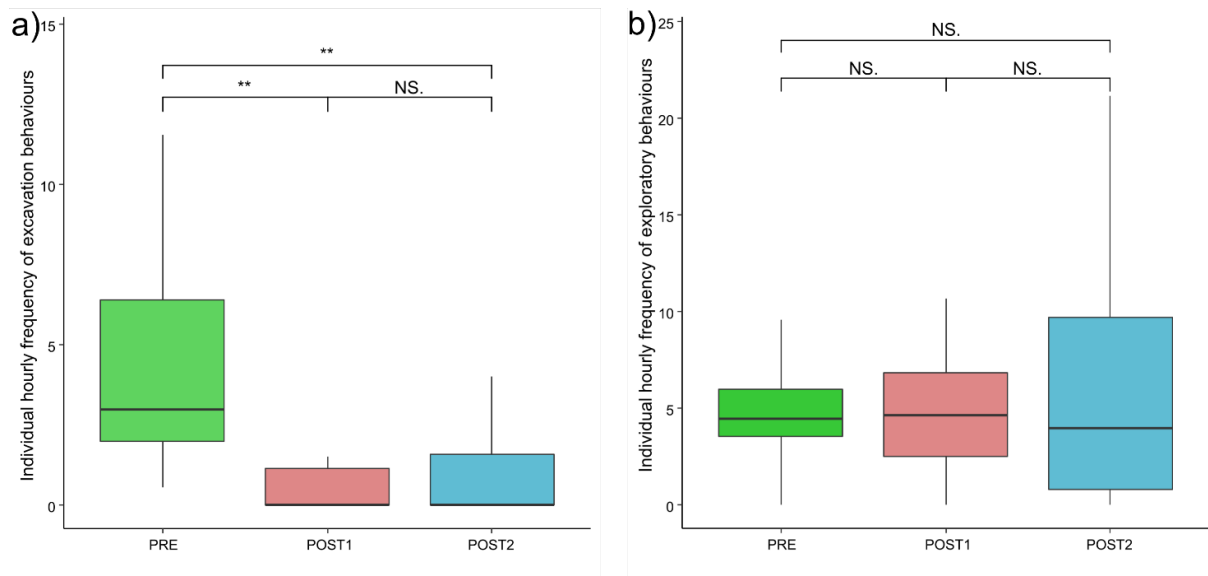


Figure 5. Box plots showing that: (a) in experimental group (EG) of free-ranging domestic pigs the levels of rooting behaviours were lower in the first week (POST1) and in the third week (POST2) after nose-clipping than in the week before nose-clipping (PRE), whereas there were no differences between the first week (POST1) and the third week after nose-clipping (POST2); (b) in experimental group (EG) of free-ranging domestic pigs there were no differences in the levels of exploratory behaviours. Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS=non-significant, **= $p < 0.01$.

In CG, we did not find any difference across the three periods in both the excavation (Friedman test, $n_{CG} = 17$, $\chi^2 = 3.169$, $df = 2$, $p = 0.205$) and exploratory activities (Friedman test, $n_{CG} = 17$, $\chi^2 = 1.156$, $df = 2$, $p = 0.561$). Therefore, in EG - but not in CG - rooting activities were lowered after nose-clip application while exploratory behaviours were not affected by nose-clips in both EG and CG.

Table 3. Summary of the results obtained from the analysis of behaviours and saliva samples of domestic pigs.

Summary of results	
Anxiety and stress	
Anxiety-related behaviours	Experimental group (Friedman test: $n_{EG} = 17$, $Mdn_{PRE} = 0.020$, $Mdn_{POST1} = 0.074$, $Mdn_{POST2} = 0.044$, $\chi^2 = 12.125$, $df = 2$, $p = 0.002$) POST1 > PRE (Dunn test: $Q = -1.118$, $p = 0.003$) POST1 > POST2 (Dunn test: $Q = 0.824$, $p = 0.049$) PRE $\approx$ POST2 (Dunn test: $Q = -0.249$, $p = 1.000$)
	Control group (Friedman test: $n_{CG} = 17$, $Mdn_{PRE} = 0.013$, $Mdn_{POST1} = 0.033$, $Mdn_{POST2} = 0.022$, $\chi^2 = 1.524$, $df = 2$, $p = 0.467$) No statistical difference across the three periods
Cortisol levels	Experimental group (Friedman test: $n_{EG} = 7$, $Mdn_{PRE} = 2.420$, $Mdn_{POST1} = 8.720$, $Mdn_{POST2} = 0.000$, $\chi^2 = 8.380$, $df = 2$, $p = 0.002$)

	POST1 > PRE (Dunn test: $Q = -1.286$, $p = 0.048$) POST1 > POST2 (Dunn test: $Q = -1.286$, $p = 0.048$) PRE $\approx$ POST2 (Dunn test: $Q = -0.000$, $p = 1.000$) Control group (Friedman test: $n_{CG} = 10$, $Mdn_{PRE} = 6.465$, $Mdn_{POST1} = 8.655$, $Mdn_{POST2} = 8.670$, $\chi^2 = 0.359$, $df = 2$, $p = 0.091$) No statistical difference across the three periods
Social domain: affiliation and aggression	
Body contacts with nose	Experimental group (Friedman test: $n_{EG} = 17$, $Mdn_{PRE} = 1.600$, $Mdn_{POST1} = 0.444$, $Mdn_{POST2} = 1.333$, $\chi^2 = 10.698$, $df = 2$, $p = 0.005$) POST1 < PRE (Dunn test: $Q = 2.915$, $p = 0.011$) POST1 < POST2 (Dunn test: $Q = -2.487$, $p = 0.039$) PRE $\approx$ POST2 (Dunn test: $Q = 0.668$, $p = 1.000$) Control group (Friedman test: $n_{CG} = 17$, $Mdn_{PRE} = 2.000$, $Mdn_{POST1} = 2.000$, $Mdn_{POST2} = 1.333$, $\chi^2 = 2.375$, $df = 2$, $p = 0.305$) No statistical difference across the three periods
Body contacts without nose	Experimental group (One-way ANOVA test: $n_{EG} = 17$, $Mean_{PRE} \pm S.E. = 2.885 \pm 0.336$, $Mean_{POST1} \pm S.E. = 2.871 \pm 0.506$, $Mean_{POST2} \pm S.E. = 2.677 \pm 0.399$, $\chi^2 = 0.077$, $df = 2$, $p = 0.926$) No statistical difference across the three periods Control group (One-way ANOVA test: $n_{CG} = 17$, $Mean_{PRE} \pm S.E. = 3.504 \pm 0.423$, $Mean_{POST1} \pm S.E. = 3.524 \pm 0.400$, $Mean_{POST2} \pm S.E. = 2.378 \pm 0.389$, $\chi^2 = 2.636$, $df = 2$, $p = 0.082$) No statistical difference across the three periods
Aggression	Experimental group (One-way ANOVA test, $n_{EG} = 17$, $Mean_{PRE} \pm S.E. = 5.099 \pm 0.825$, $Mean_{POST1} \pm S.E. = 3.157 \pm 0.575$, $Mean_{POST2} \pm S.E. = 3.123 \pm 0.548$, $\chi^2 = 2.922$, $df = 2$, $p = 0.063$) No statistical difference across the three periods Control group (Friedman test, $n_{CG} = 17$, $Mdn_{PRE} = 3.500$, $Mdn_{POST1} = 3.000$, $Mdn_{POST2} = 2.857$, $\chi^2 = 4.388$, $df = 2$, $p = 0.111$) No statistical difference across the three periods
Impact on environment	
Excavation behaviours	Experimental group (Friedman test: $n_{EG} = 17$, $Mdn_{PRE} = 3.000$, $Mdn_{POST1} = 0.000$, $Mdn_{POST2} = 0.000$, $\chi^2 = 13.935$, $df = 2$, $p = 0.001$) POST1 < PRE (Dunn test: $Q = 3.087$, $p = 0.006$) POST2 < PRE (Dunn test: $Q = 3.087$, $p = 0.006$) POST1 $\approx$ POST2 (Dunn test: $Q = 0.000$, $p = 1.000$) Control group (Friedman test, $n_{CG} = 17$, $Mdn_{PRE} = 2.500$, $Mdn_{POST1} = 1.333$, $Mdn_{POST2} = 1.333$, $\chi^2 = 3.169$, $df = 2$, $p = 0.205$) No statistical difference across the three periods

Exploratory behaviours	Experimental group (Friedman test, $n_{EG} = 17$, $Mdn_{PRE} = 4.444$, $Mdn_{POST1} = 4.667$, $Mdn_{POST2} = 4.000$, $\chi^2 = 0.567$, $df = 2$, $p = 0.753$)
	No statistical difference across the three periods
	Control group (Friedman test, $n_{CG} = 17$, $Mdn_{PRE} = 4.000$, $Mdn_{POST1} = 2.000$, $Mdn_{POST2} = 2.000$, $\chi^2 = 1.156$, $df = 2$, $p = 0.561$)
	No statistical difference across the three periods

Abbreviations: EG = Experimental group; CG = Control group; PRE = third week before the nose-clipping; POST1 = first week after the nose-clipping; POST2 = third week after the nose-clipping

5.4 Discussion

In the current study, we observed that the application of the nose-clips resulted in several outcomes: i) a transient increase in anxiety, as indicated by self-directed behaviours and stress, measured by salivary cortisol levels. These effects peaked immediately after clip application but returned to pre-application levels within the third week, thus confirming *Predictions 1a* and *1b* (**Figure 3; Table 3**); ii) a temporary decrease in levels of affiliation involving the nose, specifically nose-to-nose and nose-to-body contacts. However, these levels were restored to their original pre-clip application state within the third week, thus partially confirming *Prediction 2b* (**Figure 4a; Table 3**). No significant variation was observed in other affiliation contacts or rates of aggression, contrary to *Prediction 2a*; iii) a stable reduction of rooting, thus confirming *Prediction 3a* (**Figure 5a; Table 3**); iv) no discernible variation in the exploratory activities, contrary to *Prediction 3b* (**Figure 5b; Table 3**). In summary, the application of nose-clips appears to offer a viable compromise as it allows pigs to graze in natural areas while minimizing negative impacts on both habitat integrity and animal welfare. This solution seems to preserve the animal freedoms (McCausland, 2014), including the freedom of pigs to express most of their natural behaviours and the freedom from distress, pain, and discomfort caused by certain artificial conditions, while simultaneously mitigating environmental damage.

During the first week after the application of the nose-clips (POST1), the experimental subjects showed an increase in both anxiety-related behaviours and salivary cortisol levels compared to the control group (**Figure 3a and 3b; Table 3**). Our findings are in line with previous studies suggesting that cortisol levels increase in response to a stressor (Koopmans et al., 2005; Beerda et al., 1998; Riek et al., 2019) and that anxiety-related behaviours may be closely associated with a cortisol-mediated stress response (Beerda et al., 2000, Thompson, 2011; Kubota et al., 2014; Rinaldi, 2019). In general, pigs do not exhibit stereotypical/abnormal behaviours under extensive farming conditions and are able to implement behavioural strategies (i.e., social buffering) which allow animals to manage the consequences of stressful events by restoring anxiety to pre-event levels (Norscia et al., 2021a, b). Thus, in such breeding conditions, even if the application of nose-clips causes a temporary increase in anxiety, this could be immediately buffered by the animals through their social mechanisms. We demonstrated a transient decrease in the frequencies of affiliative

contacts involving the nose - but not in those not involving the nose - following nose-clipping (**Figure 4a and 4b; Table 3**). In previous studies (Horrell et al., 2000, 2001) the application of the nose-ring led to a decrease in nose contact with surfaces or other individuals. On the contrary in our tested pigs the reduction in affiliation involving the nose is temporary and levels of this kind of affiliative contacts were resumed after three weeks from the application of the nose-clips (**Figure 4a; Table 3**). When pigs are free to interact under natural conditions, they use their snouts for recognition and engage in social contacts involving the nose that fosters social bonds (Camerlink and Turner, 2013). In this sense, the application of the nasal clips could allow free-ranging pigs to return to engage in social behaviours that involve use of the nose after a short time and furthermore without affecting the levels of affiliative interactions not involving the nose. Previous studies showed that stress can affect levels of affiliative behaviour (Biben and Champoux 1999; Wilson 2001; Klein et al., 2010; Norscia and Palagi, 2010). In our study, the decreased levels of affiliative behaviours involving the nose were temporarily associated with increased anxiety-related behaviour levels, with the original pre-clip application levels being restored within the third week. In this regard, the increase in stress levels during the first post-clip application week (POST1) may affect negatively and specifically the levels of affiliative behaviours involving the nose.

Interestingly, in a previous study on free-ranging pigs (Norscia et al., 2021b), the level of anxiety-related behaviours was found to be significantly reduced after an affiliative interaction. In social mammals, including humans, anxiety can be buffered by affiliative interactions between subjects (i.e., social buffering; Kikisui et al., 2016; Thorsteinsson et al., 1998; Aureli and Yates, 2010; Kikisui et al., 2016) and previous studies have also found evidence that anxiety can be buffered by affiliation in the domestic pig (Reimert et al., 2014; Norscia et al., 2021b). Based on these findings, we can posit that affiliative behaviours (both involving and not involving the nose) may play a key role in restoring anxiety levels in the experimental group after nose-clipping.

As concerns aggressive behaviours, our results showed that in the experimental group the frequencies of aggressive events did not differ before and after the nose-clip application (Table 3). Higher levels of anxiety can be associated with higher levels of aggression (Rodgers et al., 1997; Ferrari et al., 1998). However, the fact that the frequency of anxiety-related behaviours and salivary cortisol levels were restored already in the third week post-clip application could explain the absence of a relevant variation in the frequencies of aggressive behaviours. Moreover, since the rank positions of the experimental subjects did not vary between PRE and POST (as indicated in the result section), the experimental group maintained their dominance relationships despite the application of nose-clips. Because aggression is the way to re-establish and change dominance hierarchy ranks (Drew, 1993), the use of aggression to restore one's rank was probably not necessary as hierarchy did not change before and after nose-clip application. According to previous studies indicating that positive social interactions can reduce tension within a group of pigs (Uvnäs-Moberg, 1998; Camerlink and Turner, 2013), our findings suggest that affiliative contacts could play an important role in reducing tension even after the transient

increase in anxiety observed during the first week after the application of the nasal clip. Hence, all types of affiliative interactions may ensure the maintenance of social relationships and group cohesion, limiting the occurrence of aggressive behaviour, as observed in previous studies in other social mammals (Marler, 1976; Lehmann et al., 2007, De Dreu 2012).

Finally, we found that the invasive rooting activities decreased immediately after the application of the nose-clips (POST1) and remained at low levels even in the third week after nose-clipping (**Figure 5a; Table 3**). Therefore, our results are in line with previous studies that showed a decrease in excavation behaviour following the application of nose-rings (Horrell et al., 2000, 2001; Studnitz et al., 2003a,b). In contrast, less invasive exploratory behaviours (grazing/browsing and foraging) did not show significant differences after the application of nose-clips (**Figure 5b; Table 3**). Rooting is a highly motivated exploratory behaviour in domestic pigs and is even considered a behavioural need (Horrell et al., 2001; Studnitz et al., 2003a, b). The domestic pig needs to perform exploratory behaviour and uses its snout to explore the environment and objects (Studnitz et al., 2007). After application of the nose-ring, pigs can replace rooting with other relevant exploratory behaviours and when ringed pigs were prevented from carrying out these exploratory activities a higher level of abnormal behaviour was observed (Studnitz et al., 2003b). However, our study showed that when pigs were unable to root, they were still able to engage in exploratory activities that did not involve the nose such as grazing/browsing and foraging. Contrary to previous studies (Studnitz et al., 2003a,b), our results do not show an increase in exploratory activities that do not involve the nose such as grazing/browsing and foraging. Therefore, we cannot assume that these behaviours can replace rooting behaviour after nose-clipping, as it did after the application of nose-rings. In our study, we recorded very few cases of behaviours such as digging with the legs or pawing that could replace the rooting behaviour after the application of the nose-rings, as suggested by previous studies (Studnitz et al., 2003a, b). In addition, previous studies have shown that nasal rings can negatively affect feeding efficiency in pigs by reducing their rooting behaviour (Horrell et al, 2000). However, the fact that at the end of our study we did not detect any difference between the experimental and control groups in relation to the individual body size (see Preliminary results) indicates that nose-clips did not affect the overall weight gain of the subjects. Hence, clipped-pigs were able to access food resources similar to non-clipped pigs. Probably, the extensive rearing conditions provided pigs with more variable and alternative food resources (other than the pellets provisioned by the farmer) that can be consumed without the use of the nose such as bark, leaves, fruits, and seeds. It has been hypothesised that the inability to perform highly motivated behaviours (such as rooting) may lead to a stress response in swine (Martínez-Miró et al., 2016). Therefore, the transient increase in both salivary cortisol levels and the frequency of anxiety-related behaviours may be affected by the inability to perform rooting behaviour. However, our results showed that anxiety levels returned to pre-application levels within the third week unlike the frequency of rooting behaviour, which remained lower than pre-clip application levels. As described above,

it is possible that other aspects including anxiety buffering mechanisms and extensive farming conditions are sufficient to restore anxiety and salivary cortisol levels. Previous studies have shown that if ringed pigs are allowed to explore the environment, individuals do not exhibit abnormal behaviour (Studnitz et al., 2003b, Studnitz et al. 2007). In line with these findings, nose-clipping in our study did not adversely affect non-invasive exploratory behaviours such as foraging, grazing, and navigation. This may also have helped to keep stress levels and anxiety-related behaviours under control.

Given the relevance of social interactions in alleviating anxiety among pigs (as observed, for example, in other groups from the same study site: Norscia et al., 2021; Cordoni et al., 2023), we cannot discount the possibility that the presence of unclipped individuals from the control group contributed to buffering anxiety more effectively than if only clipped pigs were present. Future studies may take this aspect into account and/or management measures may consider including unclipped individuals in the group. Moreover, in this study we could not consider long follow-up periods and examine measures of more chronic stress, possibly related to limited rooting or other aspects (e.g. from hair cortisol; e.g. Wiechers et al., 2021). Further investigation may consider this issue. However, caution should be taken in setting the experimental design, as the more time elapses from clip application, the more variables can come into play and cause behavioural variation. These variables may include seasonal changes with temperature/humidity fluctuations, group composition changes due to culling, individual growth and development, and possible interference with other stressors coming along the way. The removal of confounding variables may not be totally possible in open, natural spaces and may require an indoor environment. Thus, attention should be paid to the study feasibility, which is necessary to draw solid and reliable conclusions. Finally, further investigation may delve into possible differences in how males and females respond to the nose clipping procedure. In principle, one sex could be more adversely affected than the other, and, if so, management plans should take this aspect into consideration.

In conclusion, the response of pigs to the application of nose-clips appears to involve a cascade of events aimed at coping with stressful conditions and restoring homeostasis. It is essential to recognize that attempting to eliminate all stressors may lead to a reduction in both environmental stimulation and the exchange of social support, which are crucial for promoting individual resilience to stressors and maintaining the physical and psychological health of animals (Špinka, 2006; Ozbay et al., 2007; Rault, 2012). Although ideally pigs should not be prevented from rooting, to maximise their welfare, nose-clipping emerges as a mild invasive technique, effective in preserving the natural environment without altering positive social dynamics. This approach is a good compromise that allows for a balanced consideration of both animal welfare and environmental concerns.

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Ethical Statement: The present study was purely observational. The nose-clip application was carried out by the farmer as part of regular management activities and the moderate invasive procedures (i.e., size measurements and saliva samples collection) were carried out by the experimenters. The project was approved by the Bio-ethical Committee of the University of Torino (Approval nr.0615995 - 16/12/2022). The study species was not an endangered or a locally rare species. We never removed any individual from the group and we did not interact with the pigs. No aberrant or stereotypic behaviour was recorded in the study group. We took all possible precautions to minimize the impact of our presence and we carried out a habituation process during the first days of the study, even though the animals were already habituated to human presence. In order to identify each subject, we simply marked pigs with non-toxic livestock painting spray. Marking was renewed every 4-7 days depending on weather conditions. This research is part of the broader projects “So.Pig” (*Socially Pig*) and “Green Pig”.

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5.5 References

- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49, 227–266. <https://doi.org/10.1163/156853974X00534>
- Aureli, F., & Yates, K. (2010). Distress prevention by grooming others in crested black macaques. *Biology Letters*, 6, 27–29. <https://doi.org/10.1098/rsbl.2009.0513>
- Barros, M., & Tomaz, C. (2002). Non-human primate models for investigating fear and anxiety. *Neuroscience and Biobehavioral Reviews*, 26, 187–201. [https://doi.org/10.1016/S0149-7634\(01\)00064-1](https://doi.org/10.1016/S0149-7634(01)00064-1)
- Beerda, B., Schilder, M. B. H., van Hooff, J. A. R. A. M., de Vries, H. W., & Mol, J. A. (1998). Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science*, 58, 365–381. [https://doi.org/10.1016/S0168-1591\(97\)00145-7](https://doi.org/10.1016/S0168-1591(97)00145-7)
- Beerda, B., Schilder, M. B. H., van Hooff, J. A. R. A. M., de Vries, H. W., & Mol, J. A. (2000). Behavioural and hormonal indicators of enduring environmental stress in dogs. *Animal Welfare*, 9, 49–62. <https://doi.org/10.1017/S0962728600022247>
- Biben, M., & Champoux, M. (1999). Play and stress: Cortisol as a negative correlate of play in *Saimiri*. *Play and Culture Studies*, 2, 191–208.
- Birteeb, P. T., Tetteh, S. O., & Salifu, A. S. (2015). Growth performance and weight estimation of large white piglets weaned at different ages. *Research & Reviews: Journal of Veterinary Science and Technology*, 4, 15–23.
- Blackshaw, J. K., & Hagelsø, A. M. (1990). Getting-up and lying-down behaviours of loose-housed sows and social contacts between sows and piglets during Day 1 and Day 8 after parturition. *Applied Animal Behaviour Science*, 25, 61–70. [https://doi.org/10.1016/0168-1591\(90\)90070-T](https://doi.org/10.1016/0168-1591(90)90070-T)
- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, 92, 375–397. <https://doi.org/10.1016/j.physbeh.2007.02.003>
- Bourin, M., Petit-Demoulière, B., Dhonnchadha, B. N., & Hascöet, M. (2007). Animal models of anxiety in mice. *Fundamental & Clinical Pharmacology*, 21, 567–574. <https://doi.org/10.1111/j.1472-8206.2007.00526.x>
- Camerlink, I., & Reimert, I., & Bolhuis, J. E. (2016). Intranasal oxytocin administration in relationship to social behaviour in domestic pigs. *Physiology & Behavior*, 163, 51–57. <https://doi.org/10.1016/j.physbeh.2016.04.054>
- Camerlink, I., & Turner, S. P. (2013). The pig's nose and its role in dominance relationships and harmful behaviour. *Applied Animal Behaviour Science*, 145, 84–91. <https://doi.org/10.1016/j.applanim.2013.02.008>

Dantzer, R. (2002). Can farm animal welfare be understood without taking into account the issues of emotion and cognition? *Journal of Animal Science*, 80, E1–E9.

De Dreu, C. K. W. (2012). Oxytocin modulates cooperation within and competition between groups: An integrative review and research agenda. *Hormones and Behavior*, 61, 419–428. <https://doi.org/10.1016/j.yhbeh.2011.12.009>

De Vries, H., Stevens, J. M. G., & Vervaecke, H. (2006). Measuring and testing the steepness of dominance hierarchies. *Animal Behaviour*, 71, 585–592. <https://doi.org/10.1016/j.anbehav.2005.05.015>

Edgar, J. L., Mullan, S. M., Pritchard, J. C., McFarlane, U. J., & Main, D. C. (2013). Towards a 'good life' for farm animals: Development of a resource tier framework to achieve positive welfare for laying hens. *Animals*, 3, 584–605. <https://doi.org/10.3390/ani3030584>

Edge, H. L., Bulman, C. A., & Edwards, S. A. (2005). Alternatives to nose-ringing in outdoor sows: The provision of root crops. *Applied Animal Behaviour Science*, 92, 15–26. <https://doi.org/10.1016/j.applanim.2004.10.021>

Epel, E. S., Crosswell, A. D., Mayer, S. E., Prather, A. A., Slavich, G. M., Puterman, E., & Mendes, W. B. (2018). More than a feeling: A unified view of stress measurement for population science. *Frontiers in Neuroendocrinology*, 49, 146–169.

Farm Animal Welfare Council. (1996). Report on the welfare of pigs kept outdoors.

Ferrari, P. F., Palanza, P., Parmigiani, S., & Rodgers, R. J. (1998). Interindividual variability in Swiss male mice: Relationship between social factors, aggression, and anxiety. *Physiology & Behavior*, 63(5), 821–827. [https://doi.org/10.1016/S0031-9384\(97\)00544-1](https://doi.org/10.1016/S0031-9384(97)00544-1)

Goumon, S., Illmann, G., Leszkowová, I., Dostalová, A., & Cantor, M. (2020). Dyadic affiliative preferences in a stable group of domestic pigs. *Applied Animal Behaviour Science*, 230, 105045. <https://doi.org/10.1016/j.applanim.2020.105045>

Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2, 186. <https://doi.org/10.12966/abc.05.08.2014>

Horrell, R. I. (1992). Effects of environmental enrichment on growing pigs. *Proceedings of the British Society of Animal Production*, 1992, 134–134. <https://doi.org/10.1017/S0308229600022455>

Horrell, R. I., A'Ness, P. J., Edwards, S. A., & Riddoch, I. (2000). Nose-rings influence feeding efficiency in pigs. *Animal Science*, 71(2), 259–264. <https://doi.org/10.1017/S1357729800055090>

Horrell, R. I., A'Ness, P. J., Edwards, S. A., & Eddison, J. C. (2001). The use of nose-rings in pigs: Consequences for rooting, other functional activities, and welfare. *Animal Welfare*, 10, 3–22.

- Jensen, P. (1986). Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 16(2), 131–142. [https://doi.org/10.1016/0168-1591\(86\)90105-X](https://doi.org/10.1016/0168-1591(86)90105-X)
- Kells, N. J. (2022). Review: The Five Domains model and promoting positive welfare in pigs. *Animal*, 16(Suppl 2), 100378. <https://doi.org/10.1016/j.animal.2021.100378>
- Kikusui, T., Winslow, J. T., & Mori, Y. (2006). Social buffering: Relief from stress and anxiety. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1476), 2215–2228. <https://doi.org/10.1098/rstb.2006.1941>
- Klein, Z. A., Padow, V. A., & Romeo, R. D. (2010). The effects of stress on play and home cage behaviors in adolescent male rats. *Developmental Psychobiology*, 52(1), 62–70. <https://doi.org/10.1002/dev.20413>
- Kleszcz, A., Cholewińska, P., Front, G., Pacoń, J., Bodkowski, R., Janczak, M., & Dorobisz, T. (2022). Review on selected aggression causes and the role of neurocognitive science in the diagnosis. *Animals*, 12(3), 281. <https://doi.org/10.3390/ani12030281>
- Koopmans, S. J., Ruis, M., Dekker, R., van Diepen, H., Korte, M., & Mroz, Z. (2005). Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. *Physiology & Behavior*, 85(4), 469–478. <https://doi.org/10.1016/j.physbeh.2005.05.010>
- Kristensen, H. H., Jones, R. B., Schofield, C. P., White, R. P., & Wathes, C. M. (2001). The use of olfactory and other cues for social recognition by juvenile pigs. *Applied Animal Behaviour Science*, 72(4), 321–333. [https://doi.org/10.1016/S0168-1591\(00\)00209-4](https://doi.org/10.1016/S0168-1591(00)00209-4)
- Kubota, N., Amemiya, S., Yanagita, S., Nishijima, T., & Kita, I. (2014). Emotional stress evoked by classical fear conditioning induces yawning behavior in rats. *Neuroscience Letters*, 566, 182–187. <https://doi.org/10.1016/j.neulet.2014.02.064>
- Lehmann, J., Korstjens, A. H., & Dunbar, R. I. M. (2007). Group size, grooming, and social cohesion in primates. *Animal Behaviour*, 74(6), 1617–1629. <https://doi.org/10.1016/j.anbehav.2006.10.025>
- Leiva, D., & De Vries, H. (2022). Testing Steepness of Dominance Hierarchies. *R-project.org*. Retrieved January 12, 2023, from <https://cran.r-project.org/web/packages/steepness/>
- Marino, L., & Colvin, C. M. (2015). Thinking pigs: A comparative review of cognition, emotion, and personality in *Sus domesticus*. *International Journal of Comparative Psychology*, 28, 1–27. <https://doi.org/10.46867/ijcp.2015.28.00.04>
- Marler, P. (1976). On animal aggression: The roles of strangeness and familiarity. *The American Psychologist*, 31(3), 239–246. <https://doi.org/10.1037/0003-066X.31.3.239>
- Martínez-Miró, S., Tecles, F., Ramón, M., Escribano, D., Hernández, F., Madrid, J., Orengo, J., Martínez-Subiela, S., Manteca, X., & Cerón, J. J. (2016). Causes, consequences and biomarkers of stress in swine: An update. *BMC Veterinary Research*, 12, 171. <https://doi.org/10.1186/s12917-016-0791-8>

McCausland, C. (2014). The five freedoms of animal welfare are rights. *Journal of Agricultural & Environmental Ethics*, 27(4), 649–662. <https://doi.org/10.1007/s10806-013-9483-6>

McLeman, M., Mendl, M., Jones, R. B., & Wathes, C. M. (2004). Bimodal and unimodal discrimination of individual conspecifics by juvenile domestic pigs. In *Proceedings of the 38th International Congress of the ISAE* (Eds. Hänninen, L., & Valros, A.), Helsinki, Finland, pp. 118–118.

Mellor, D. J. (2015a). Positive animal welfare states and reference standards for welfare assessment. *New Zealand Veterinary Journal*, 63(1), 17–23. <https://doi.org/10.1080/00480169.2014.926802>

Mellor, D. J. (2015b). Enhancing animal welfare by creating opportunities for positive affective engagement. *New Zealand Veterinary Journal*, 63(1), 3–8. <https://doi.org/10.1080/00480169.2014.926799>

Mendl, M., Randle, K., & Pope, S. (2002). Young female pigs can discriminate individual differences in odours from conspecific urine. *Animal Behaviour*, 64(1), 97–101. <https://doi.org/10.1006/anbe.2002.3040>

Miller, L. J., & Chinnadurai, S. K. (2023). Beyond the five freedoms: Animal welfare at modern zoological facilities. *Animals*, 13(11), 1818. <https://doi.org/10.3390/ani13111818>

Mundry, R., & Fischer, J. (1998). Use of statistical programs for nonparametric tests of small samples often leads to incorrect p-values: Examples from animal behaviour. *Animal Behaviour*, 56(2), 256–259. <https://doi.org/10.1006/anbe.1998.0756>

Murase, K., Watanabe, T., Arai, S., Kim, H., Tohya, M., Ishida-Kuroki, K., Võ, T. H., Nguyễn, T. P. B., Nakagawa, I., Osawa, R., Nguyễn, N. H., & Sekizaki, T. (2019). Characterization of pig saliva as the major natural habitat of *Streptococcus suis* by analyzing oral, fecal, vaginal, and environmental microbiota. *PLOS ONE*, 14(4), e0215983. <https://doi.org/10.1371/journal.pone.0215983>

Mutua, F. K., Dewey, C. E., Arimi, S. M., Schelling, E., & Ogara, W. O. (2011). Prediction of live body weight using length and girth measurements for pigs in rural Western Kenya. *Journal of Swine Health and Production*, 19(1), 26–33.

Neumann, I. D., Veenema, A. H., & Beiderbeck, D. I. (2010). Aggression and anxiety: Social context and neurobiological links. *Frontiers in Behavioral Neuroscience*, 4, 928. <https://doi.org/10.3389/fnbeh.2010.00012>

Norscia, I., Coco, E., Robino, C., Chierito, E., & Cordoni, G. (2021a). Yawn contagion in domestic pigs (*Sus scrofa*). *Scientific Reports*, 11, 1851. <https://doi.org/10.1038/s41598-020-80545-1>

Norscia, I., Collarini, E., & Cordoni, G. (2021b). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>

Norscia, I., & Palagi, E. (2011). When play is a family business: Adult play, hierarchy, and possible stress reduction in common marmosets. *Primates*, 52(2), 101–104. <https://doi.org/10.1007/s10329-010-0228-0>

Ozbay, F., Johnson, D. C., Dimoulas, E., Morgan, C. A., Charney, D., & Southwick, S. (2007). Social support and resilience to stress: From neurobiology to clinical practice. *Psychiatry*, 4(5), 35–40.

Panksepp, J. (2005). Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition*, 14(1), 30–80. <https://doi.org/10.1016/j.concog.2004.10.004>

Paschek, N., Müller, N., Heistermann, M., Ostner, J., & Schülke, O. (2019). Subtypes of aggression and their relation to anxiety in Barbary macaques. *Aggressive Behavior*, 45(2), 120–128. <https://doi.org/10.1002/ab.21801>

Porges, S. W. (2003). Social engagement and attachment: A phylogenetic perspective. *Annals of the New York Academy of Sciences*, 1008(1), 31–47. <https://doi.org/10.1196/annals.1301.004>

Puppe, B., Ernst, K., Schön, P. C., & Manteuffel, G. (2007). Cognitive enrichment affects behavioural reactivity in domestic pigs. *Applied Animal Behaviour Science*, 105(1–3), 75–86. <https://doi.org/10.1016/j.applanim.2006.05.016>

Rault, J. L. (2012). Friends with benefits: Social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science*, 136(1), 1–14. <https://doi.org/10.1016/j.applanim.2011.10.002>

Rault, J. L. (2019). Be kind to others: Prosocial behaviours and their implications for animal welfare. *Applied Animal Behaviour Science*, 210, 113–123. <https://doi.org/10.1016/j.applanim.2018.10.015>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology & Behavior*, 129, 221–229. <https://doi.org/10.1016/j.physbeh.2014.02.059>

Riek, A., Schrader, L., Zerbe, F., & Petow, S. (2019). Comparison of cortisol concentrations in plasma and saliva in dairy cattle following ACTH stimulation. *The Journal of Dairy Research*, 86(4), 406–409. <https://doi.org/10.1017/S0022029919000669>

Rinaldi, G. (2019). The itch-scratch cycle: A review of the mechanisms. *Dermatology Practical & Conceptual*, 9(2), 90–97. <https://doi.org/10.5826/dpc.0902a03>

Rodgers, R. J., Cao, B. J., Dalvi, A., & Holmes, A. (1997). Animal models of anxiety: An ethological perspective. *Brazilian Journal of Medical and Biological Research*, 30(3), 289–304. <https://doi.org/10.1590/s0100-879x1997000300002>

Sandi, C., & Haller, J. (2015). Stress and the social brain: Behavioural effects and neurobiological mechanisms. *Nature Reviews Neuroscience*, 16(5), 290–304. <https://doi.org/10.1038/nrn3918>

- Špinka, M. (2006). How important is natural behaviour in animal farming systems? *Applied Animal Behaviour Science*, 100(2), 117–128. <https://doi.org/10.1016/j.applanim.2006.04.006>
- Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science*, 48(3), 419–425. <https://doi.org/10.1017/S0003356100040411>
- Stookey, J. M., & Gonyou, H. W. (1998). Recognition in swine: Recognition through familiarity or genetic relatedness? *Applied Animal Behaviour Science*, 55(3–4), 291–305. [https://doi.org/10.1016/S0168-1591\(97\)00046-4](https://doi.org/10.1016/S0168-1591(97)00046-4)
- Studnitz, M., Jensen, K. H., & Jørgensen, E. (2003a). The effect of nose rings on the exploratory behaviour of outdoor gilts exposed to different tests. *Applied Animal Behaviour Science*, 84(1), 41–57. [https://doi.org/10.1016/S0168-1591\(03\)00144-8](https://doi.org/10.1016/S0168-1591(03)00144-8)
- Studnitz, M., Jensen, K. H., Jørgensen, E., & Jensen, K. K. (2003b). The effect of nose ringing on exploratory behaviour in gilts. *Animal Welfare*, 12(1), 109–118.
- Studnitz, M., Jensen, M. B., & Pedersen, L. J. (2007). Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. *Applied Animal Behaviour Science*, 107(3–4), 183–197. <https://doi.org/10.1016/j.applanim.2006.11.013>
- Thompson, S. B. N. (2011). Born to yawn? Cortisol linked to yawning: A new hypothesis. *Medical Hypotheses*, 77(5), 861–862. <https://doi.org/10.1016/j.mehy.2011.07.056>
- Thorsteinsson, E. B., James, J. E., & Gregg, M. E. (1998). Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology*, 17(5), 436–444. <https://doi.org/10.1037/0278-6133.17.5.436>
- Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress*, 5(1), 47–54. <https://doi.org/10.1080/102538902900012378>
- Uvnäs-Moberg, K. (1998). Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology*, 23(8), 819–835. [https://doi.org/10.1016/S0306-4530\(98\)00056-0](https://doi.org/10.1016/S0306-4530(98)00056-0)
- Wiechers, D. H., Brunner, S., Herbrandt, S., Kemper, N., & Fels, M. (2021). Analysis of hair cortisol as an indicator of chronic stress in pigs in two different farrowing systems. *Frontiers in Veterinary Science*, 8, 605078. <https://doi.org/10.3389/fvets.2021.605078>
- Wilson, J. H. (2001). Prolactin in rats is attenuated by conspecific touch in a novel environment. *Cognitive, Affective & Behavioral Neuroscience*, 1(2), 199–205. <https://doi.org/10.3758/cabn.1.2.199>

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6. Balancing welfare and habitat damage in pigs (*Sus scrofa*) under extensive farming: an ethological approach for determining the effects of group composition and environmental enrichment

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Abstract

According to the modern perspective on evaluating animal welfare, it is important to consider both negative and positive experiences. This study investigated the impact of group composition and environmental enrichments on the behaviours of free-ranging pigs, focusing on anxiety-related behaviours, aggression, affiliation, post-conflict affiliation, excavation, and non-invasive exploration activities. Data were collected on three different groups (all-female: n =10; all-male: n =13; mixed-sex: n =12) of free-ranging pigs raised in a natural woodland habitat at the ethical farm “Parva Domus” (Turin, Italy). To evaluate the impact of environmental enrichment, further data collection was carried out on the mixed-sex group under three different enrichment conditions (absence; dry leaves; straw) provided in a rotational scheme. Group composition did not affect levels of anxiety-related behaviours, aggression, and non-invasive exploration. However, it did impact other social aspects (affiliation: One-way ANOVA: $p = 0.003$; post-conflict affiliation: Kruskal–Wallis: $p = 0.005$). In particular, the mixed-sex and the all-male groups showed higher levels of affiliation than the all-female group and the mixed-sex group showed higher levels of post-conflict affiliation than the all-male group. Moreover, we found differences in excavation behaviour levels (Kruskal–Wallis: $p = 0.001$), with higher levels of excavation behaviour in the mixed-sex group compared to the all-female group. Regarding the impact of enrichments, we found differences in levels of anxiety-related behaviours (One-way ANOVA: $p = 0.046$), affiliation (One-way ANOVA: $p = 0.006$), excavation (One-way

ANOVA: $p < 0.001$), and non-invasive exploration activities (One-way ANOVA: $p < 0.001$). In enrichment conditions with straw, we found a significant decrease in anxiety-related, affiliation, and excavation behaviours levels, and a significant increase in non-invasive exploration behaviours. A trend with lower levels of aggression was observed in straw enrichment condition compared to the absence of enrichment, although the difference was not significant. Moreover, there were no differences in post-conflict affiliation frequencies. Our findings suggest that forming mixed-sex groups and providing environmental enrichments such as the straw could be suitable solutions to effectively reduce invasive excavation behaviours without preventing pigs from expressing their natural behavioural repertoire, thus maintaining high standards of animal welfare. This study highlights behavioural aspects to be considered in extensive farming, confirming the importance of ethology as a tool for assessing pig welfare.

6.1 Introduction

The ethology plays a significant role in the increase of animal welfare. Understanding the behavioural repertoire of a given species permits to enhance its well-being as in the case of farm-raised animals (Kokocińska and Kaleta, 2016). By knowing animal behavioural norms, at least their basic needs can be addressed. Ethological studies represent the primary step in evaluating animal physical, social, and behavioural conditions (Dellmeier, 1989; Temple et al., 2011). According to the modern definition, animal welfare does not simply rely on the absence of negative events, but also on the presence of positive experiences (Mellor, 2015; Mellor and Beausoleil, 2015; Špinka, 2017; Lawrence et al., 2018). Therefore, both negative and positive behaviours have to be considered when assessing animal welfare. In case of farm-raised animals, a good management taking into account animal welfare can enhance and ameliorate food production and sustainability (Appleby and Mitchell, 2018).

Domestic pigs are social animals (Stolba and Wood-Gush, 1989) and, under extensive farming conditions, they can behaviourally express anxiety (not related to stereotypical behaviours) via a set of displacement activities, which can be considered as a proxy of stress (e.g., vacuum chewing, self-scratching/rubbing, yawning, body shaking; Norscia et al., 2021; Collarini et al., under review¹). However, pigs are also able to cope with anxiety by exchanging affiliative contacts such as body contact and grooming (social buffering; Norscia et al., 2021, 2024). After an aggression occurring over a resource, pigs may engage in post-conflict affiliative behaviours (i.e. reconciliation, solicited and unsolicited triadic contacts and quadratic contacts) to possibly restore group homeostasis (Cordoni et al., 2023; Norscia et al., 2024). In a welfare assessment perspective, it is important to consider that positive social behaviour promotes cohesion and affiliation among conspecifics, as well as reduces the negative effects of stress (Temple et al., 2011). Excavation and exploration are other activities that can improve pig welfare, even though these behaviours cause damage to the natural habitat (Day et al., 1995; Edge et al., 2005; Studnitz et al., 2007).

Environmental enrichments are a widely employed solution to enhance the welfare of pigs, and previous studies confirm the benefits derived from the use of environmental enrichment (Ernst et al., 2005; Godyń et al., 2019). Specifically, enriching the pig environment is a way to reduce pathological behaviours (including aggression and stereotypies), promoting the expression of positive behaviours (EFSA Panel on Animal Health and Welfare (AHAW), 2014; Godyń et al., 2019). Furthermore, group composition and/or environmental enrichments (e.g. straw, vegetation parts) can mitigate negative situations and enhance positive behaviours (Day et al., 2001; Van de Weerd et al., 2003; Studnitz et al., 2007).

In this study, we took into account behavioural parameters to assess which conditions might buffer negative events and possibly enhance positive states. Specifically, we investigated whether different group compositions (all males, all

females, and mixed-sex groups) and enrichments (none, dry leaves or straw, for the mixed-sex group) could influence levels of negative (anxiety-related behaviours, aggression, and excavation) and positive (affinitive behaviours, post-conflict strategies, and non-invasive exploration) behaviours. Here below we describe our predictions in detail.

6.1.1 Predictions

Under natural conditions, pig groups are composed by either female or young male associations and can freely interact with vegetation (Stolba and Wood-Gush, 1989; Gabor et al., 1999; D'Eath and Turner, 2008; Gonyou, 2001). Feral pigs form strong social bonds, particularly with related individuals, and aggression levels are very limited (Podgórski et al., 2014). In domestic pigs, establishing a stable social hierarchy could help avoid future conflicts and promote good social cohesion (Arey and Edwards, 1998; D'Eath and Turner, 2008). Moreover, an unstable social environment can lead to increased stress levels (Cunha et al., 2018). Because pigs do not naturally form mixed-sex groups, we expected that in the mixed-sex group (artificially established and possibly less stable) compared to other group compositions we would find the highest level of negative events (anxiety-related behaviours, aggression, and excavation; *prediction 1, Table 1*) and the lowest level of buffering behaviours (affinitive behaviours, post-conflict strategies, and non-invasive exploration; *prediction 2, Table 1*). Previous studies in pigs have shown that a sterile environment increases the risk of aggressive behaviours and stress (Petersen et al., 1995; Moinard et al., 2003; Brown et al., 2018). Furthermore, the presence of enrichments can promote exploratory behaviours and positive social interactions among individuals (Vanheukelom et al., 2012). Therefore, we expected that in the absence of the environmental enrichment compared to other conditions we would find the highest level of negative events (anxiety-related behaviours, aggression, and excavation; *prediction 3, Table 1*) and the lowest level of buffering behaviours (affinitive behaviours, post-conflict strategies, and non-invasive exploration; *prediction 4, Table 1*).

Table 1. Management factors and conditions considered in this study and related predictions

Management factor	Negative welfare	Positive welfare
	<i>Prediction 1</i>	<i>Prediction 2</i>
Group composition (all males, all females, or mixed-sex group)	In mixed-sex group (artificially established but not naturally found) compared to other group compositions we expected the highest level of: a) anxiety-related behaviours	In the mixed-sex group (artificially established but not naturally found) compared to other group compositions we expected the lowest level of: a) affiliation b) post-conflict

	b) aggression, and c) excavation behaviours	affinitive behaviours, and c) exploration behaviours
Enrichment for mixed-sex group (none, straw, or leaves)	Prediction 3	Prediction 4
	In absence of environmental enrichment compared to other conditions, we expected the highest level of: a) anxiety-related behaviours b) aggression, and c) excavation behaviours	In absence of environmental enrichment compared to other conditions, we expected the lowest level of: a) affiliation b) post-conflict affinitive behaviours, and c) exploration behaviours

6.2 Materials and Methods

6.2.1 Study groups

This study was conducted from January to March 2022 on three different groups of free-ranging domestic pigs (*Sus scrofa*) at the Ethical Farm “Parva Domus” located at Cavagnolo (Turin, Italy). Each group was housed in a 3-ha fenced enclosure. The enclosures were located within an area covered by natural woodland habitat, and the groups were not in visual or acoustic contact with each other, despite sharing the same habitat conditions. The study groups consisted of i) a group of sexually mature females composed by 10 adult females (7–15 months; all-female group; hereafter AFg); ii) a group composed by 13 castrated young males (all-male group; hereafter AMg; 7–10 months of age) and iii) a mixed-sex group composed by 12 individuals of similar age (mixed-sex group; hereafter MSg) including 6 sexual mature females (7–10 months of age) and 6 castrated young males (7–10 months of age). All individuals were of three mixed-breeds (not pure-breed as at least one grandparent belonged to a different breed): Parma Black, Large White, and Piedmont Black. Moreover, all individuals had the same sire (mixed-breed), thus they could be either full siblings or half siblings. Males had been castrated via the removal of testes within their first days of life.

Each study group was formed 2 weeks before the beginning of the study to ensure group stability by the beginning of data collection. The pigs were provided with food (Ciclo Unico P, SILDAMIN®) every day from 9:30 to 10:30 and water was available *ad libitum*. The subjects could supplement their food intake with roots, leaves, fruits, seeds, and bark naturally available in the environment. For individual recognition, the pigs were marked with spray Raidex® for livestock, used by the farmers to mark animals, with no manipulation because the pigs were habituated to the human presence.

6.2.2 Data collection and operational definitions

Data were gathered via focal animal sampling (Altmann, 1974), which consisted of collecting all behaviours (audio recording) performed by one individual at a time within a predetermined interval (15 minutes), recording their behaviours using continuous recording (all occurrences within the interval). Focal samplings were carried out on all individuals in each group ($n_{AFg} = 10$; $n_{AMg} = 13$; $n_{MSg} = 12$) and following a rotational scheme, we blindly selected the focal pig from a pool of pig identification codes. Data collection was conducted on a daily basis from 08:30 am to 05:30 pm.

Effects of group composition. Data collection followed a rotation of time slots (randomly selected, excluding those already covered) during which the behaviour of the focal animal was sampled. To ensure a balance in observations, multiple focal samplings were conducted for each individual throughout the data collection period at different times of day and each individual in each group was observed for 6 hr. We collected data on anxiety-related (vacuum-chewing, scratching/body-rubbing, head/body-shaking, and yawning; Norscia et al., 2021), affiliative and aggressive behaviours, post-conflict affiliation (affiliative contacts following in the three minutes after an aggressive event; Cordoni et al., 2023; Norscia et al., 2024), excavation (rooting plus rasping and pawing), and non-invasive exploration (grazing/browsing plus foraging). The behaviours were categorised following the ethogram used by Studnitz et al. (2003), Norscia et al. (2021, 2024) and Cordoni et al. (2023). Audio recordings were carried out by E.C., A.P. and L.C. by voice recording application for smartphone (O.S. Android). Before starting systematic data collection I.N. and G.C. supervised E.C., A.P. and L.C. in a training period during which inter-observer reliability on Cohen's k was determined for anxiety-related, aggression, affiliative, post-conflict, excavation, and exploratory behaviours (Cohen's $0.87 \leq k \leq 0.92$).

To reduce possible biases due to changes in temperature, humidity, and climatic conditions, observations were carried out during the same period.

Effects of enrichment conditions. Further data collection (27 days) was carried out by E.C. on the MSg to assess the effect of different enrichment conditions on the frequencies of the behaviours described above (aggression, affiliation, anxiety behaviours, post-conflict affiliation, excavation; exploration). During this period of data collection, three different conditions of environmental enrichments were provided to the group: no enrichment (hereafter NE; **Figure 1a**); dry leaves (*Quercus petraea*, *Quercus pubescens*, *Carpinus betulus*, *Sambucus nigra*, *Robinia pseudoacacia*; hereafter LE; **Figure 1b**) and straw (hereafter ST; **Figure 1c**).



Figure 1. Photos representing the three conditions of environmental enrichment: **(a)** absence of environmental enrichment, **(b)** dry leaves and **(c)** straw. Individuals of the mixed-sex group are visible.

We applied a study design by dividing a period of 27 days into three different time blocks of 9 days each. Following a rotation scheme, all three different enrichment conditions were provided for each block (9 days). Each condition was maintained for three consecutive days. To avoid potential biases and to assess that the condition of the forest floor did not influence the interactions with the added enrichments, we included the no enrichment condition as a control in the experimental design. To rotate the different environmental enrichments, we randomly picked the conditions for each 9-day time block by blindly selecting the name from a conditions pool, using three cards (each one with a different enrichment condition) to be extracted manually from a bag. The random extraction of conditions followed a non-replacement sampling technique, thereby excluding conditions that had already been drawn within the 9-day block (**Figure 2**). Environmental enrichment was provided every morning at 8:30 A.M. The amount of daily enrichment was always the same (10kg divided into 4 bags; each bag contained 2.5kg of environmental enrichment; bag capacity = 56l; **Figure 3**) and was supplied each time in the same way at the same points (**Figure 1**). Distribution was not clumped to avoid conflict over concentrated material spots. Moreover, data collection followed a rotation of time slots (randomly selected, excluding those already

covered) during which the behaviour of the focal animal was sampled. As for comparison across groups, to ensure a balance in observations, multiple focal samplings were conducted for each individual throughout the data collection period at different times of day and each individual was observed for a total of 18 hr (6 hr in NE condition; 6 hr in LE condition and 6 hr in ST condition).

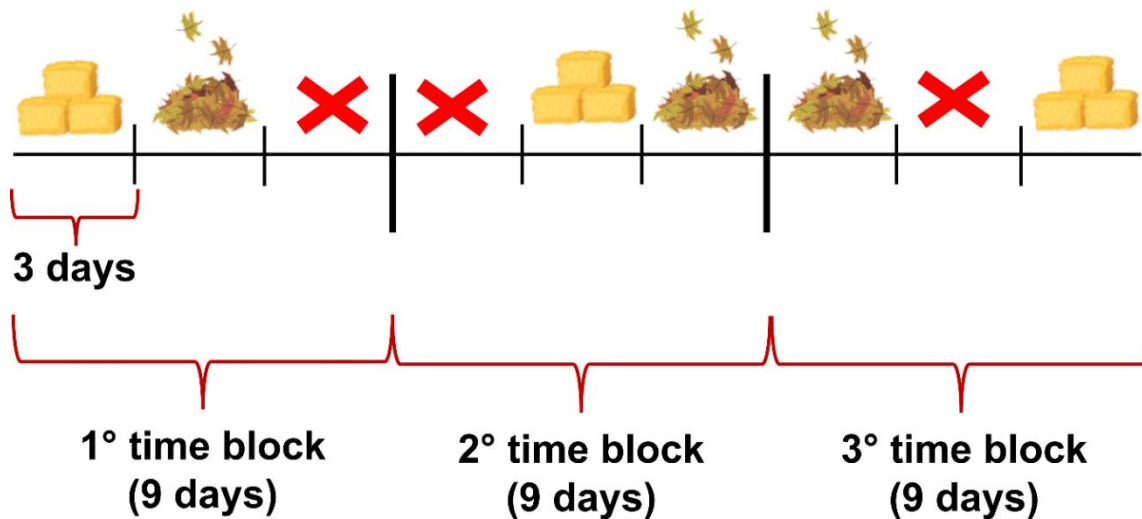


Figure 2. Rotation scheme of the three environmental enrichment conditions.



Figure 3. Photos representing (a) a bag containing dry leaves and (b) a bag containing straw. Each bag contained 2.5kg of environmental enrichment; bag capacity = 56l.

Anxiety-related behaviours. We considered as anxiety-related behaviours the patterns described by Norscia et al. (2021): yawning, head/body shaking, scratching/body rubbing, and vacuum chewing. These behavioural patterns are associated with stressful events (under natural conditions; Norscia et al., 2021) and their fluctuation can follow the fluctuation of cortisol levels (Collarini et al., under review¹). Moreover, to compare individual levels of anxiety-related behaviours across groups and across environmental enrichment conditions, we calculated the individual absolute frequencies.

Aggression: We considered as aggression all the agonistic interactions in which one or more individuals perform one or more aggressive patterns (aggressive bite, aggressive chase, aggressive push, aggressive head knocking, Norscia et al., 2021; Cordoni et al., 2023) towards another individual. For each individual of each group, we calculated the number of aggression in which the selected individual was involved. Moreover, to compare the individual levels of aggression across groups, we normalised the number of aggression per individual on the number of group members ($N - 1$ for each group). To compare individual levels of aggression across environmental enrichment conditions, we calculated the individual absolute frequencies.

Affinitive interactions. In the analyses we considered the following affinitive behaviours: rest in contact, grooming, contacts with nose (e.g. nose–nose contacts; nose–body contacts) and head-over (Norscia et al., 2021, 2024; Cordoni et al., 2023). To compare the individual levels of affiliation across groups, we normalised the number of affinitive interactions per individual on the number of group members ($N - 1$ for each group). To compare individual levels of affiliation across environmental enrichment conditions, we calculated the individual absolute frequencies.

Post-conflict affinitive interactions. We considered as post-conflict affinitive contacts all the affinitive contacts occurring within three minutes following an aggression (Cordoni et al., 2023; Norscia et al., 2024). In particular, within a 3-min time window following an aggression, we recorded the first post-conflict affinitive contact that occurred between i) the aggressor and the aggressee (i.e. reconciliation; de Waal and van Roosmalen, 1979), ii) a third individual not involved in the previous conflict and the aggressor/aggressee (i.e. solicited and unsolicited triadic contacts; Cordoni et al., 2023), and iii) two or more individuals not involved in the conflict (i.e. quadratic contacts; Norscia et al., 2024). In the analyses we considered the following affinitive behaviours: rest in contact, grooming, contacts with nose (e.g. nose–nose contacts; nose–body contacts) and head-over (Norscia et al., 2021, 2024; Cordoni et al., 2023). For group comparison purpose, we normalised the total number of post-conflict affinitive contacts in which a selected individual was involved on the total number of aggression occurring in its group. Furthermore, to compare individual post-conflict affiliation levels across the three different conditions of environmental enrichment (no enrichment: NE; dry leaves: LE; straw: ST), we normalised the total number of post-

conflict affiliative contacts in which a selected individual was involved on the total number of aggression occurring in each condition of environmental enrichment.

Excavation and exploratory activities. We included rooting, rasping with the snout, and pawing as part of the excavation activity. For exploratory activity, we considered grazing, browsing, and foraging (we excluded those cases in which the food was provided by the farmer). Moreover, to compare individual levels of excavation and exploration behaviours across groups and across environmental enrichment conditions, we calculated the individual absolute frequencies.

6.2.3 Statistical analyses

Comparison across groups. As a first step, we performed a preliminary analysis to verify that the groups were comparable in terms of age. Owing to the non-normal data distributions (Lilliefors-corrected Kolmogorov–Smirnov test: for ages, $p \leq 0.005$; for post-conflict, excavation and exploratory behaviours, $0.001 \leq p \leq 0.200$), we applied non-parametric statistics (Siegel and Castellan, 1988). In particular, we applied Kruskal–Wallis test for k-independent samples to compare the age of individuals between the three groups (AFg; AMg; MSg) and the individual frequencies of post-conflict affiliative contacts and excavation and exploratory behaviours between the study groups. We applied the Dunn *post hoc* test for pairwise comparisons, with the significance level of probability adjusted downward using the Bonferroni correction. Owing to the normal data distributions (Lilliefors-corrected Kolmogorov–Smirnov test: $p \geq 0.05$), we applied the parametric one-way ANOVA test for k-independent samples, to compare the individual frequencies of anxiety-related behaviours and affiliative and aggressive interactions between the three groups (AFg; AMg; MSg). We did not include post-conflict affiliative contacts in this analysis (see below). We applied the Bonferroni *post hoc* test for pairwise comparisons.

Comparison across enrichment conditions. Owing to the non-normal data distributions (Lilliefors-corrected Kolmogorov–Smirnov test: $0.001 \leq p \leq 0.200$), we applied non-parametric statistics (Siegel and Castellan, 1988). In particular, we applied the non-parametric Friedman test for k-dependent samples to compare the individual frequencies of aggression and post-conflict affiliative interactions across the different enrichment conditions (NE, LE, ST). We applied the Dunn *post hoc* test for pairwise comparisons, with the significance level of probability adjusted downward using the Bonferroni correction.

For all the analyses, the statistical significance was set at $p < 0.05$. A trend of significance was considered for discussion for $0.05 \leq p < 0.1$.

6.3 Results

6.3.1 Comparison across groups

Preliminary analysis showed that the individuals of the all-female (AFg), all-male (AMg), and mixed-sex (MSg) groups had similar ages (Kruskal–Wallis test: $\chi^2 = 5.131$, $df = 2$, $p = 0.077$; mean age $\pm$ SE: AFg = 10.90 ± 1.12 , AMg = 8.61 ± 0.43 ; MSg = 8.00 ± 0.43).

The frequencies of anxiety-related behaviours did not show any significant difference between the three groups (One-way ANOVA test: $F = 1.014$; $df = 2$; $p = 0.374$; mean value $\pm$ SE: AFg = 16.70 ± 3.46 , AMg = 18.38 ± 2.66 ; MSg = 13.25 ± 1.91 ; **Figure 4a**). Similarly, the frequencies of aggression did not show any significant difference between the groups (One-way ANOVA test: $\chi^2 = 0.738$; $df = 2$; $p = 0.486$; mean value $\pm$ SE: AFg = 0.39 ± 0.09 , AMg = 0.50 ± 0.05 ; MSg = 0.41 ± 0.08 ; **Figure 4b**). Hence, group composition affected neither anxiety behaviours nor aggression levels.

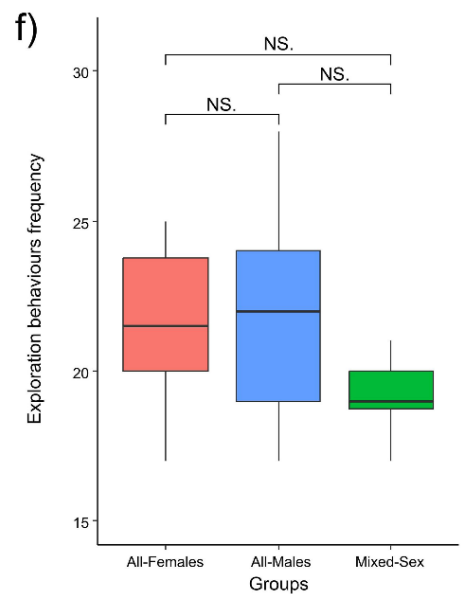
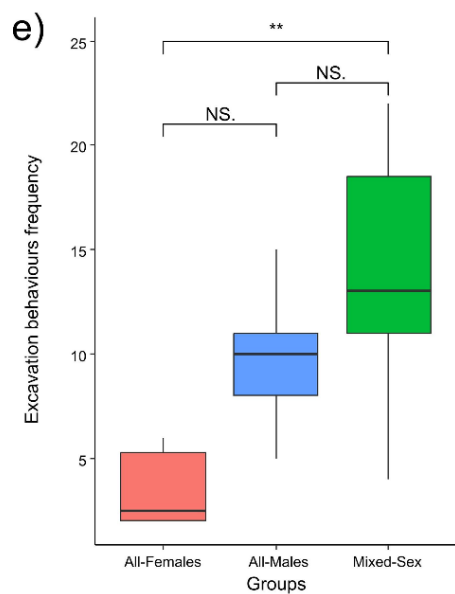
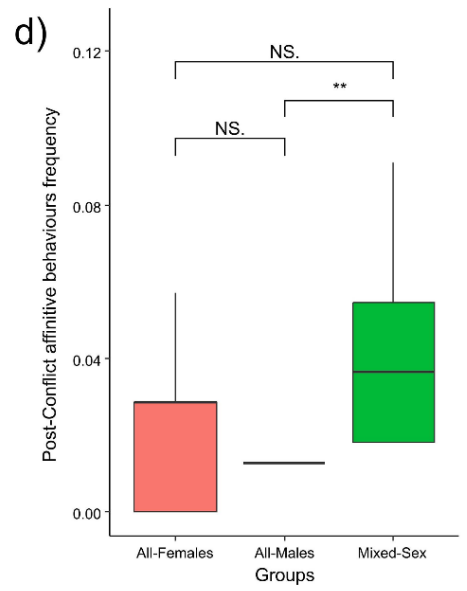
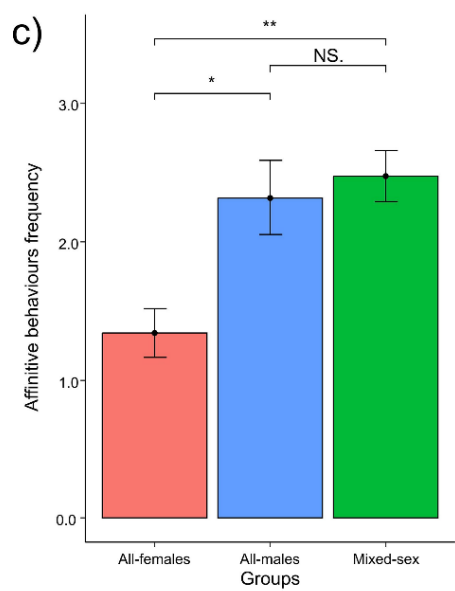
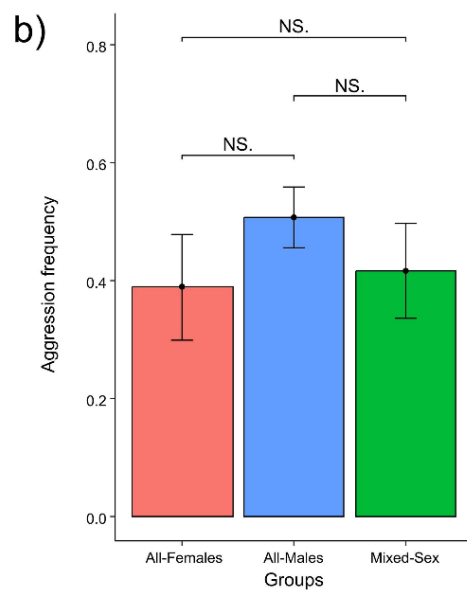
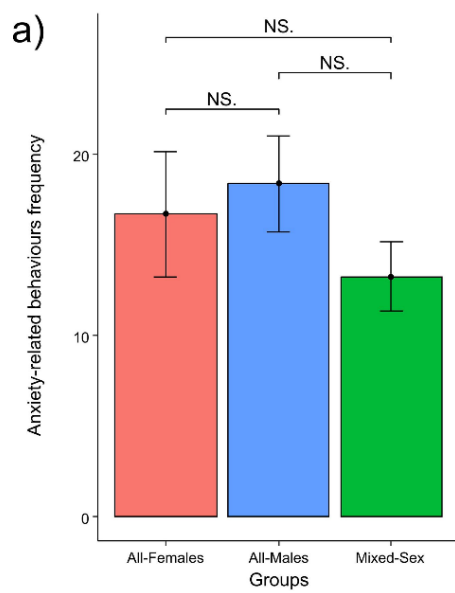


Figure 4. Comparisons across groups: **(A)** error bar plots showing that there were no differences in levels of anxiety-related behaviours across groups (One-way ANOVA test; $p = 0.374$); **(B)** error bar plots showing that there were no differences in levels of aggression (normalised on the number of group members) across groups (One-way ANOVA test; $p = 0.486$); **(C)** error bar plots showing differences in levels of affiliative behaviours (normalised on the number of group members) across groups (One-way ANOVA test, $p = 0.007$; Bonferroni *post-hoc* test, AFg vs. AMg: $p = 0.009$; AFg vs. MSg: $p = 0.026$; AFg vs. MSg: $p = 1.000$); **(D)** boxplots showing differences in levels of post-conflict affiliative behaviours (normalised on the total number of aggression occurred in the group) across groups (Kruskal–Wallis test, $p = 0.005$; Dunn *post-hoc* test, AMg vs. MSg: $p = 0.004$; AFg vs. AMg: $p = 0.970$; AFg vs. MSg: $p = 0.128$); **(E)** boxplots showing differences in levels of excavation behaviours across groups (Kruskal–Wallis test, $p = 0.001$; Dunn *post-hoc* test, MSg vs. AFg: $p = 0.001$; AFg vs. AMg: $p = 0.183$; AMg vs. MSg: $p = 0.137$); **(F)** boxplots showing that there were no differences in levels of exploration behaviours across groups (Kruskal–Wallis test, $p = 0.114$). Error bars: 95% confidence interval (bars) around the mean (dots); Boxplot: median value (horizontal line), interquartile range (box); minimum and maximum values in the data (vertical line). NS = non-significant, * = $p < 0.05$; ** = $p < 0.01$.

The frequencies of affiliative interactions varied significantly across groups (One-way ANOVA test: $F = 6.993$; $df = 2$; $p = 0.003$; mean value $\pm$ SE: AFg = 1.34 ± 0.18 , AMg = 2.32 ± 0.26 ; MSg = 2.48 ± 0.19 , **Figure 4c**). In particular, we found that the AFg showed lower affiliative interaction frequencies than AMg (Bonferroni *post-hoc* test: AFg vs. AMg; $p = 0.013$) and MSg (Bonferroni *post-hoc* test: AFg vs. MSg; $p = 0.004$), whereas we found no difference between AMg and MSg (Bonferroni *post-hoc* test: AFg vs. MSg; $p = 1.000$). Hence, the mixed-sex and all-male groups showed higher affiliation levels than the all-female group.

The frequencies of post-conflict affiliative contacts varied significantly across groups (Kruskal–Wallis test: $\chi^2 = 10.569$, $df = 2$, $p = 0.005$; mean value $\pm$ SE: AFg = 0.03 ± 0.01 , AMg = 0.01 ± 0.00 ; MSg = 0.05 ± 0.01 ; **Figure 4d**). In particular, we found that the AMg showed lower post-conflict affiliative contact frequencies than MSg (Dunn *post-hoc* test: AMg vs. MSg; $p = 0.004$), whereas we found no difference between AFg and AMg (Dunn *post-hoc* test: AFg vs. AMg; $p = 0.970$) and between AFg and MSg (Dunn *post-hoc* test: AFg vs. MSg; $p = 0.128$). Thus, the mixed-sex group showed higher levels of post-conflict affiliation compared to all-male group.

The frequencies of excavation behaviours varied significantly across the groups (Kruskal–Wallis test: $\chi^2 = 13.805$, $df = 2$, $p = 0.001$; mean value $\pm$ SE: AFg = 4.90 ± 1.48 , AMg = 9.38 ± 0.90 ; MSg = 14.25 ± 1.55 , **Figure 4e**). In particular, we found that the MSg showed higher excavation behaviour frequencies than AFg (Dunn *post-hoc* test: MSg vs. AFg; $p = 0.001$), whereas we found no difference between AFg and AMg (Dunn *post-hoc* test: AFg vs. AMg; $p = 0.183$) and AMg and MSg (Dunn *post-hoc* test: AMg vs. MSg; $p = 0.137$). Hence, the mixed-sex group showed higher excavation frequencies, compared to the all-female group.

The frequencies of non-invasive exploration behaviours did not show any significant difference between the three groups (Kruskal–Wallis test: $\chi^2 = 4.339$, $df = 2$, $p = 0.114$; mean value $\pm$ SE: AFg = 21.40 ± 0.93 , AMg = 21.69 ± 0.94 ; MSg =

19.33 ± 0.45 ; **Figure 4f**). Hence, the level of non-invasive exploration was not affected by group composition.

6.3.2 Comparison of different environmental enrichment conditions

The frequencies of anxiety-related behaviours varied significantly across the different conditions of environmental enrichments (One-way ANOVA test: $F = 3.385$; $df = 2$; $p = 0.046$; mean value $\pm$ SE: NE = 13.08 ± 2.07 , LE = 11.25 ± 1.83 ; ST = 7.00 ± 1.01 ; **Figure 5a**). In particular, we found lower frequencies of anxiety related-behaviours in ST than in NE condition (Bonferroni *post-hoc* test: ST vs. NE; $p = 0.048$), whereas we found no difference between LE and ST conditions (Bonferroni *post-hoc* test: LE vs. ST; $p = 0.257$) and LE and NE conditions (Bonferroni *post-hoc* test; LE vs. NE; $p = 1.000$). Thus, only ST condition was associated with reduced anxiety behaviours compared to NE.

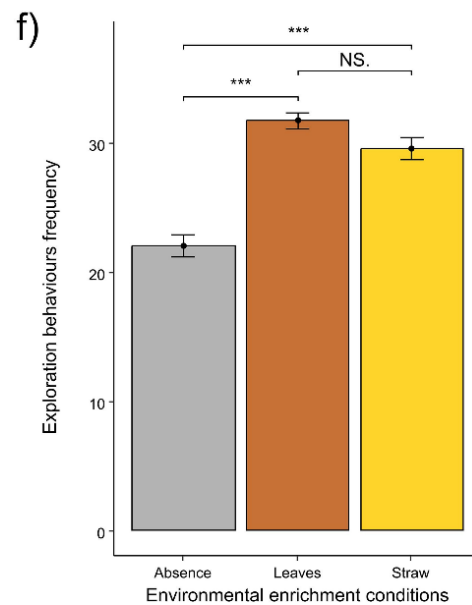
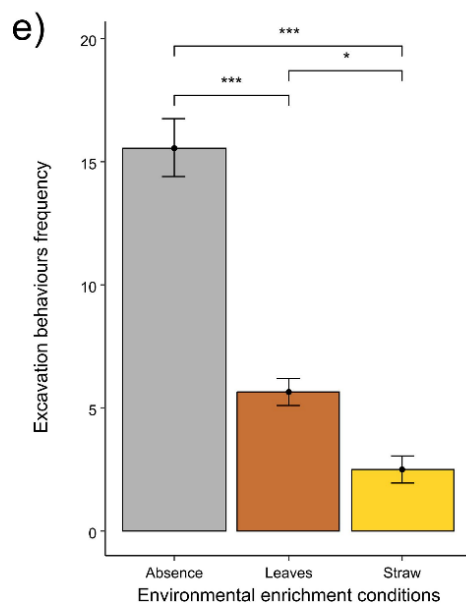
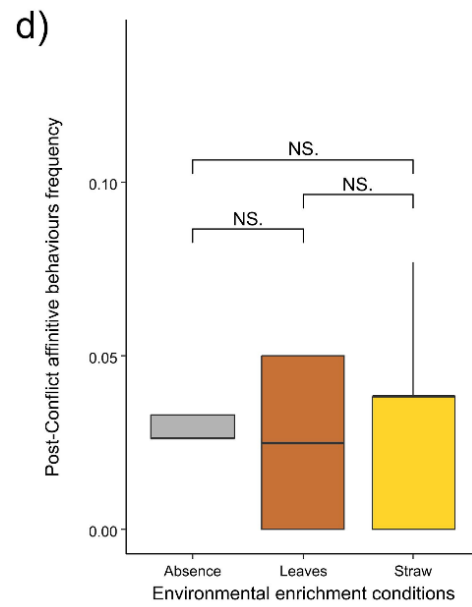
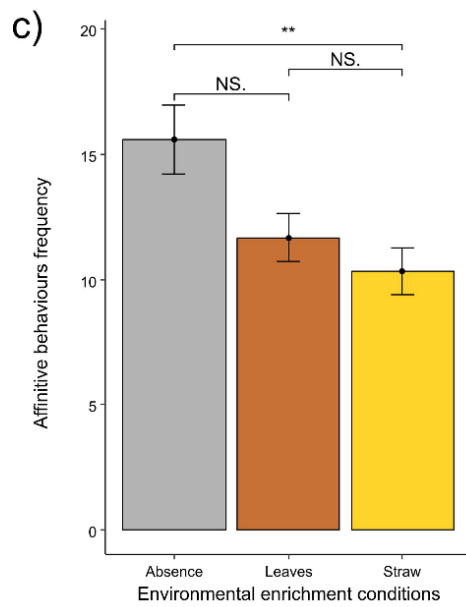
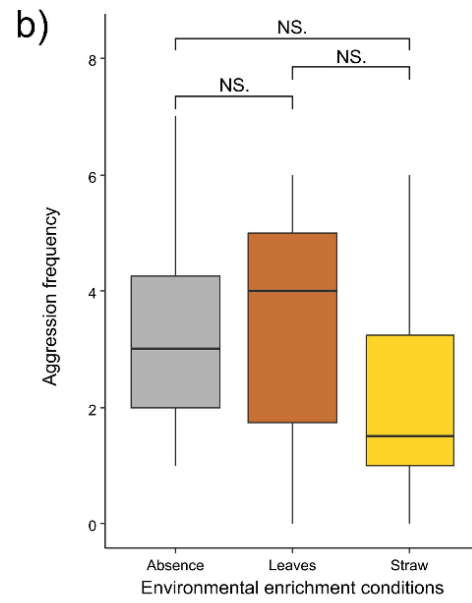
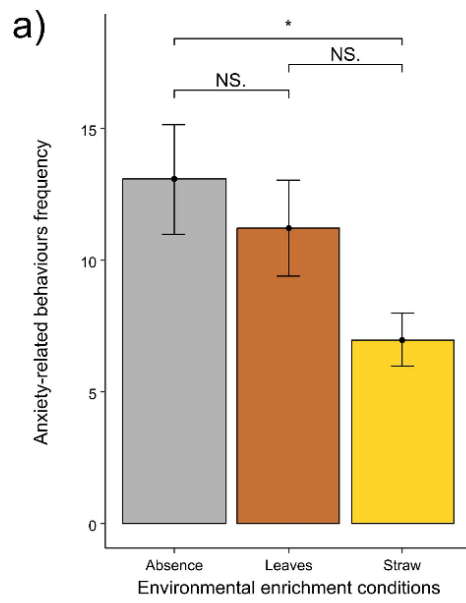


Figure 5. Comparison across environmental enrichment conditions: **(A)** error bar plots showing differences in levels of anxiety-related behaviours across environmental conditions (One-way ANOVA test, $p = 0.046$; Bonferroni *post-hoc* test; ST vs. NE: $p = 0.048$; LE vs. ST: $p = 0.257$; LE vs. NE: $p = 1.000$); **(B)** boxplots showing differences in levels of aggression across environmental conditions (Friedman test $p = 0.040$; Dunn *post-hoc* test, ST vs. LE: $p = 0.124$; LE vs. NE: $p = 1.000$; ST vs. NE: $p = 0.074$); **(C)** error bar plots showing differences in levels of affiliative behaviours across environmental conditions (One-way ANOVA test, $p = 0.006$; Bonferroni *post-hoc* test, ST vs. NE: $p = 0.006$; LE vs. ST: $p = 1.000$; LE vs. NE: $p = 0.053$); **(D)** boxplots showing that there were no differences in levels of post-conflict affiliative behaviours (normalised on the total number of attacks occurred in the same condition of environmental enrichment) across environmental conditions (Friedman test: $p = 0.929$); **(E)** error bar plots showing differences in levels of excavation behaviours across environmental conditions (One-way ANOVA test, $p < 0.001$; Bonferroni *post-hoc* test, ST vs. LE: $p = 0.030$; ST vs. NE: $p < 0.001$; LE vs. NE: $p < 0.001$); **(F)** error bar plots showing differences in levels of exploration behaviours across environmental conditions (One-way ANOVA test, $p < 0.001$; Bonferroni *post-hoc* test, ST vs. NE: $p < 0.001$; LE vs. NE: $p < 0.001$; LE vs. ST: $p = 0.178$). Error bars: 95% confidence interval (bars) around the mean (dots); Boxplot: median value (horizontal line), interquartile range (box); minimum and maximum values in the data (vertical line). NS = non-significant, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

The frequencies of aggression varied significantly across conditions (Friedman test: $\chi^2 = 6.435$, $df = 2$, $p = 0.040$; mean value $\pm$ SE: NE = 3.16 ± 0.52 , LE = 3.33 ± 0.62 ; ST = 2.17 ± 0.51 ; **Figure 5b**). However, the pairwise comparisons showed any significant difference (Dunn *post-hoc* test: ST vs. LE: $Q = 2.041$, $p = 0.124$; LE vs. NE: $Q = 0.204$, $p = 1.000$; ST vs. NE: $Q = 2.245$, $p = 0.074$) Hence, none enrichment condition had a significant effect on aggression frequency, although straw tended to be associated with reduced conflict levels.

The frequencies of affiliative interactions showed significant difference across conditions (One-way ANOVA test: $F = 6.065$; $df = 2$; $p = 0.006$; mean value $\pm$ SE: NE = 15.59 ± 1.38 , LE = 11.67 ± 0.96 ; ST = 10.33 ± 0.93 ; **Figure 5c**). In particular, we found lower frequencies of affiliative behaviours in ST than in NE condition (Bonferroni *post-hoc* test: ST vs. NE; $p = 0.006$), whereas we found no difference between LE and ST (Bonferroni *post-hoc* test: LE vs. ST; $p = 1.000$) and a statistical trend between LE and NE conditions (Bonferroni *post-hoc* test: LE vs. NE; $p = 0.053$). Hence, straw was associated with reduced affiliation compared to no enrichment condition.

The frequencies of post-conflict affiliative contacts did not show any significant difference across conditions (Friedman test: $\chi^2 = 0.146$, $df = 2$, $p = 0.929$; mean value $\pm$ SE: NE = 0.04 ± 0.01 , LE = 0.02 ± 0.01 ; ST = 0.03 ± 0.01 ; **Figure 5d**).

The frequencies of excavation behaviours showed significant variation across conditions (One-way ANOVA test: $F = 69.731$; $df = 2$; $p < 0.001$; mean value $\pm$ SE: NE = 15.58 ± 1.17 , LE = 5.67 ± 0.57 ; ST = 2.50 ± 0.56 ; **Figure 5e**). In particular, we found lower frequencies of excavation behaviours in ST than in both LE (Bonferroni *post-hoc* test: ST vs. LE; $p = 0.030$) and NE (Bonferroni *post-hoc* test: ST vs. NE; $p < 0.001$) and in LE than in NE conditions (Bonferroni *post-hoc* test: LE vs. NE; $p < 0.001$). Thus, both straw and leaves were associated with reduced excavation frequencies but straw was the most effective enrichment in this respect.

The frequencies of non-invasive exploration behaviours significantly differed across conditions (One-way ANOVA test: $F = 41.818$; $df = 2$; $p < 0.001$; mean value $\pm$ SE: NE = 22.00 ± 0.87 , LE = 31.67 ± 0.61 ; ST = 29.50 ± 0.85 ; **Figure 5f**). In particular, we found higher frequencies of exploration behaviours in ST than in NE (Bonferroni *post-hoc* test: ST vs. NE; $p < 0.001$) and in LE than in NE conditions (Bonferroni *post-hoc* test: LE vs. NE; $p < 0.001$), whereas we found no difference between LE and ST conditions (Bonferroni *post-hoc* test: LE vs. ST; $p = 0.178$). Hence, both straw and leaves were associated with an increase in non-invasive exploratory behaviours.

6.4 Discussion

Our results show that group composition (all-male, all-female, and mixed-sex groups) did not affect the level of anxiety, aggression, and non-invasive exploration. However, group composition seemed to affect other aspects of social life. Particularly, the mixed-sex group was associated with an increase in the levels of affiliation (as it occurred with the all-male group, compared to the all-female group), post-conflict affiliative contact (especially, compared to all-male group), and excavation activity (especially, compared with the all-female group). Focusing on the mixed-sex group and different enrichment conditions, we found no effect on post-conflict affiliation levels but straw was associated with i) reduced anxiety and affiliative behaviour levels (compared to when no enrichment was provided) and possibly with reduced aggression levels (we obtained a statistical trend); ii) lowest levels of excavation, and iii) (as it occurred with leaves) enhanced non-invasive exploration activity.

6.4.1 Comparison across groups

The lack of effect of group composition on aggression levels may be due to different factors including group stability and farming conditions. In the wild, pig groups have a relatively stable composition and they are composed by related adult females with their offspring or by young males (Stolba and Wood-Gush, 1989; Gabor et al., 1999; D'Eath and Turner, 2008; Gonyou, 2001). In our case, the three study groups were formed well before the beginning of the observations and no individual was replaced during data collection. From this perspective, we may assume that our groups were stable. The stability of group composition can reduce aggression; indeed, high levels of aggression can be observed in groups where individuals are regularly replaced (Simmins, 1993; Lagoda et al., 2021; Jowett and Amory, 2021). Moreover, it has been observed that pigs can develop preferred social relationships, and such social preferences are weakly influenced by individual attributes, including sex, but are predominantly influenced by the behavioural context (Goumon et al., 2020). Furthermore, we have to take into account the space available to pigs. Increased space availability may promote a reduction in aggressive event frequencies in the long-term; where space is virtually unlimited the levels of aggression are very low (Jensen and Wood-Gush, 1984; Arey and Edwards, 1998). Moreover, since castration may reduce

aggression in males (Fredriksen et al., 2008), another aspect to consider is that males were castrated (both in the all males and mixed-sex groups) and this may have helped to keep the levels of aggression under control in groups where males were present. Hence, extensive farming conditions, group stability and male castration may help keeping aggression under control in all study groups regardless of group composition.

Intra-group aggression is one of the major sources of anxiety in social mammals (Aureli et al., 2002), and no difference in aggression levels between the three groups may also account for the absence of differences in anxiety-related behaviours. Moreover, the extensive farming conditions – rather than the composition of the groups- could play an important role in keeping anxiety levels under control in all groups. Indeed, a previous study on another pig group housed in the same environmental condition as the present research, showed that levels of anxiety-related behaviours in free-ranging pigs were low, with an increase related to aggression, and did not differ between castrated males and females (Norscia et al., 2021).

When studying animal welfare, it is necessary to consider not only the absence of negative behaviours/events (such as anxiety and aggressive interactions) but also – and predominantly – the presence of positive interactions, such as affiliative behaviours (Bradburn, 1969; Seligman and Csikszentmihalyi, 2000). In pigs, affiliative and exploratory behaviours are considered as indicators of a positive welfare (Špinková, 2017; Lawrence et al., 2018). Regarding non-invasive exploratory behaviours, our results showed no differences across groups. In line with previous studies that have shown that pigs raised in semi-natural conditions spend most of their time in exploratory activities (Stolba and Wood-Gush, 1989; Day et al., 1995; Studnitz et al., 2007), extensive farming conditions could play an important role in maintaining high levels of exploratory behaviour, regardless of group composition. Affiliative behaviours levels (including post-conflict interactions) were higher in the mixed-sex group than in the all-male group. In social mammals, affiliative behaviours play an important role in maintaining group homeostasis (de Waal, 2000) and in domestic pigs, these behaviours can be used to buffer anxiety (Norscia et al., 2021). Unlike the groups formed by related females and by young males that show a stable composition (Stolba and Wood-Gush, 1989; Gabor et al., 1999; D'Eath and Turner, 2008; Gonyou, 2001), the mixed groups, composed of females and castrated males, could be less stable from a social point of view, as these groups are artificially established and not found naturally. In this regard, the higher levels of affiliative behaviours observed in the mixed group may be necessary due to the potential lower stability of the composition and contribute effectively to maintaining the social cohesion of the group, promoting the maintenance of group homeostasis. Moreover, the absence of variations in the levels of anxiety-related behaviours across groups may suggest that high levels of affiliative behaviours could help keep anxiety under control especially in the mixed group, where males and females share space and resources.

From a welfare point of view, it is important to consider not only the frequency of aggression but even if the aggression are managed with efficient post-conflict

strategies and our results showed that the levels of post-conflict affiliative interactions were higher in the mixed-sex group than in the all-male group. Aggressive events are an integral part of social life (de Waal, 2000) and previous studies showed that domestic pigs can effectively engage in post-conflict strategies (e.g. reconciliation, triadic and quadratic contacts; Cordoni et al., 2023; Norscia et al., 2024). Differences in agonistic behaviour between sexes have been observed in domestic pigs, with males differing significantly from females in their fighting ability, competitive behaviour and related costs (Camerlink et al., 2022) and castrated males may also engage in aggressive behaviours for dominance (Foister et al., 2018), although we cannot exclude that castration may help reduce levels of aggression. However, aggressive interactions between castrated males and females could still be unbalanced, even though the males are castrated. Therefore, there may be a greater need for social buffering to restore social homeostasis within the mixed-sex group. Furthermore, to explain the levels of post-conflict affiliative behaviour exhibited by individuals in the mixed-sex group, it may be necessary to consider the potential social instability that could characterise the mixed-sex group, as we hypothesised for affiliative behaviours in general. In this regard, both affiliative behaviours and post-conflict affiliative behaviours could contribute to maintaining social cohesion in the mixed-sex group more than in groups formed by females or castrated males. The fact that the mixed-sex group showed the same level of aggression but more solved conflicts and higher levels of affiliative behaviours compared to the other groups could represent a positive welfare trait that should be considered in the management of free-ranging pigs.

Finally, the mixed-sex group showed higher excavation levels than the all-female group. In this regard, the presence of males within the group could play an important role, although they are castrated males. Taking into account the deep differences in agonistic behaviour between the sexes and that aggression between males are costly (Camerlink et al., 2022), indirect forms of competition could emerge to avoid conflicts. Moreover, it is worth considering that castrated males can maintain competition, although in a less aggressive way (Fredriksen et al., 2008; Foister et al., 2018). The reduction of aggression over resources (contest competition) may be associated with an increase in indirect forms of competition over food, such as scramble competition, which occurs when an individual reduces the quantity of food available to others by consuming it (Koenig, 2002). Thus, excavation may be a way to prevent others to take food resource (e.g. roots, tubers), avoiding aggressive interaction.

6.4.2 Comparison of different environmental enrichment conditions

Pigs have an innate propensity for socialization, exploration and chewing and poorly enriched environments can cause stress and induce abnormal, stereotypical and aggressive behaviours (Day et al., 2001; Studnitz et al., 2007). Environmental enrichment is one mean to reduce such behaviours, since the lack of a substrate or object to be manipulated in the environment in which pigs live can lead to an increased

frequency of pathological patterns (EFSA Panel on Animal Health and Welfare (AHAW), 2014). It has been suggested that environmental enrichment for pigs to be effective should be manipulable, chewable and edible (Van de Weerd et al., 2003).

Environmental enrichment is effective in lowering levels of aggressive behaviours (Van de Weerd et al., 2003; Bolhuis et al., 2005). Consistently, our results showed that the presence of straw tended to decrease levels of aggression compared to the no enrichment condition, although we did not observe significant variations. Extensive farming conditions could play an important role, keeping levels of aggression under control regardless of enrichment conditions. Moreover, low levels of aggression could ensure that no differences emerge in post-conflict affiliation levels, as in our case.

In line with previous studies which showed that pigs raised in sterile environments experienced more stress than in enriched environments (Van de Weerd et al., 2003), we found a reduction in anxiety-related behaviours in straw enrichment condition compared to no enrichment condition. In addition, since affiliation is an important tool to buffer anxiety (Norscia et al., 2021; Cordoni et al., 2023; Norscia et al., 2024), we could assume a cascade effect that could lead to reduced affiliation levels when anxiety levels are low. In this regard, low levels of affiliative behaviours in the straw enrichment condition may be associated with low levels of anxiety observed in the same condition. In our study, straw was an effective enrichment in lowering excavation levels and increasing levels of non-invasive exploratory behaviours. Outdoor pigs often perform rooting or foraging behaviour; rooting is an important part of the behavioural repertoire, constituting a rewarding experience and satisfying a behavioural need for pigs (Day et al., 1995; Studnitz et al., 2003, 2007). Consistent with our findings, pigs can adapt their feeding behaviour to the prevailing conditions (Gustafsson et al., 1999) and environmental enrichment can increase the time spent in explorative behaviours (Beattie et al., 2000). In our case, individuals could spend more time chewing and manipulating straw, engaging in straw exploratory behaviours rather than in excavation behaviours with negative consequences on environment.

6.4.3 Conclusion

The group composition and the type of environmental enrichment can represent two important elements for improving pig welfare while reducing animal impact on the natural habitat. In this regard, ethological studies represent an important tool for evaluating the welfare of farm-raised animals such as domestic pigs. Our study provides insight into behavioural aspects worthy of consideration for the management of extensive farming in terms of animal welfare. In particular, the mixed-sex group showed higher levels of behaviours that may indicate positive animal welfare states, such as affiliative and post-conflict affiliative behaviours, although it also exhibited higher levels of excavation behaviours compared to both all-female and all-male groups. In this regard, affiliative and post-conflict affiliative contacts could contribute to the cohesion and stability of mixed-sex groups. Moreover, this aspect could be

supported by the absence of differences regarding negative welfare aspects such as aggression and anxiety-related behaviours. From this perspective, the formation of mixed groups by farmers could be a management strategy that could enhance animal welfare. Under straw enrichment conditions, individuals exhibited lower levels of anxiety-related behaviours compared to the no enrichment condition. Conversely, in the absence of enrichment, we recorded higher levels of affiliative behaviours, which could indicate a greater need to alleviate stress compared to the straw enrichment condition. Specifically, providing straw to free-ranging pigs raised in semi-natural conditions can represent an environmental enrichment strategy that reduces negative welfare aspects while also decreasing invasive excavation behaviours, leading to an increase in non-invasive exploratory behaviours. Furthermore, our findings suggest that forming mixed-sex groups and providing them with environmental enrichment such as straw could be a suitable solution to effectively reduce invasive excavation behaviours without preventing pigs from showing their natural behavioural repertoire. Indeed, the combination of these two measures could ensure high standards of welfare, considering both the negative and positive effects, and a reduction of the impact on the natural environment.

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6.5 References

- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49(3), 227–266. <https://doi.org/10.1163/156853974x00534>
- Appleby, M. C., & Mitchell, L. A. (2018). Understanding human and other animal behaviour: Ethology, welfare and food policy. *Applied Animal Behaviour Science*, 205, 126–131. <https://doi.org/10.1016/j.applanim.2018.05.032>
- Arey, D. S., & Edwards, S. A. (1998). Factors influencing aggression between sows after mixing and the consequences for welfare and production. *Livestock Production Science*, 56(1), 61–70. [https://doi.org/10.1016/s0301-6226\(98\)00144-4](https://doi.org/10.1016/s0301-6226(98)00144-4)
- Aureli, F., Cords, M., & van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: A predictive framework. *Animal Behaviour*, 64(3), 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Beattie, V. E., O'Connell, N. E., & Moss, B. W. (2000). Influence of environmental enrichment on the behaviour, performance, and meat quality of domestic pigs. *Livestock Production Science*, 65(1–2), 71–79. [https://doi.org/10.1016/s0301-6226\(99\)00179-7](https://doi.org/10.1016/s0301-6226(99)00179-7)
- Bolhuis, J. E., Schouten, W. G. P., Schrama, J. W., & Wiegant, V. M. (2005). Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. *Applied Animal Behaviour Science*, 93(3–4), 213–228. <https://doi.org/10.1016/j.applanim.2005.01.006>
- Bradburn, N. M. (1969). *The structure of psychological well-being*. Aldine Publishing.
- Brown, S. M., Bush, S. J., Summers, K. M., Hume, D. A., & Lawrence, A. B. (2018). Environmentally enriched pigs have transcriptional profiles consistent with neuroprotective effects and reduced microglial activity. *Behavioural Brain Research*, 350, 6–15. <https://doi.org/10.1016/j.bbr.2018.05.015>
- Camerlink, I., Farish, M., Arnott, G., & Turner, S. P. (2022). Sexual dimorphism in ritualized agonistic behaviour, fighting ability, and contest costs of *Sus scrofa*. *Frontiers in Zoology*, 19, 12. <https://doi.org/10.1186/s12983-022-00458-9>
- Cordoni, G., Comin, M., Collarini, E., Robino, C., Chierto, E., & Norscia, I. (2023). Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: Cognitive and functional implications. *Animal Cognition*, 26(4), 687–701. <https://doi.org/10.1007/s10071-022-01688-4>
- Cunha, E. C. P., de Alcantara Menezes, T., Bernardi, M. L., Mellagi, A. P. G., da Rosa Ulguim, R., Wentz, I., et al. (2018). Reproductive performance, offspring characteristics, and injury scores according to the housing system of gestating gilts. *Livestock Science*, 210, 59–67. <https://doi.org/10.1016/j.livsci.2018.02.008>

D'Eath, R. B., & Turner, S. P. (2008). The natural behaviour of the pig. In *The Welfare of Pigs* (pp. 13–45). Springer Netherlands.

Day, J. E. L., Kyriazakis, I., & Lawrence, A. B. (1995). The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. *Applied Animal Behaviour Science*, 42(3), 193–206. [https://doi.org/10.1016/0168-1591\(95\)93889-9](https://doi.org/10.1016/0168-1591(95)93889-9)

Day, J. E. L., Spooler, H. A. M., & Edwards, S. A. (2001). Straw as environmental enrichment: Which properties do growing pigs find behaviourally rewarding? In Proceedings of the International Symposium of the C.I.G.R. *Animal Welfare Considerations in Livestock Housing Systems* (pp. 157–167). Newcastle University.

Dellmeier, G. R. (1989). Motivation in relation to the welfare of enclosed livestock. *Applied Animal Behaviour Science*, 22(1–2), 129–138. [https://doi.org/10.1016/0168-1591\(89\)90049-x](https://doi.org/10.1016/0168-1591(89)90049-x)

de Waal, F. B. M. (2000). Primates—a natural heritage of conflict resolution. *Science*, 289(5479), 586–590. <https://doi.org/10.1126/science.289.5479.586>

de Waal, F. B. M., & van Roosmalen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioral Ecology and Sociobiology*, 5(1), 55–66. <https://doi.org/10.1007/bf00302695>

Edge, H. L., Bulman, C. A., & Edwards, S. A. (2005). Alternatives to nose-ringing in outdoor sows: The provision of root crops. *Applied Animal Behaviour Science*, 92(1–2), 15–26. <https://doi.org/10.1016/j.applanim.2004.10.021>

EFSA Panel on Animal Health and Welfare (AHAW). (2014). Scientific Opinion concerning a multifactorial approach on the use of animal and non-animal-based measures to assess the welfare of pigs. *EFSA Journal*, 12(5), 3702. <https://doi.org/10.2903/j.efsa.2014.3702>

Ernst, K., Puppe, B., Schön, P. C., & Manteuffel, G. (2005). A complex automatic feeding system for pigs aimed to induce successful behavioural coping by cognitive adaptation. *Applied Animal Behaviour Science*, 91(3–4), 205–218. <https://doi.org/10.1016/j.applanim.2004.10.010>

Foister, S., Doeschl-Wilson, A., Roehe, R., Arnott, G., Boyle, L., & Turner, S. (2018). Social network properties predict chronic aggression in commercial pig systems. *PLOS ONE*, 13(10), e0205122. <https://doi.org/10.1371/journal.pone.0205122>

Fredriksen, B., Lium, B. M., Marka, C. H., Mosveen, B., & Nafstad, O. (2008). Entire male pigs in farrow-to-finish pens—Effects on animal welfare. *Applied Animal Behaviour Science*, 110(3–4), 258–268. <https://doi.org/10.1016/j.applanim.2007.04.007>

Gabor, T. M., Hellgren, E. C., Van Den Bussche, R. A., & Silvy, N. J. (1999). Demography, sociospatial behaviour and genetics of feral pigs (*Sus scrofa*) in a semi-arid environment. *Journal of Zoology*, 247(3), 311–322. <https://doi.org/10.1111/j.1469-7998.1999.tb00994.x>

Godyń, D., Nowicki, J., & Herbut, P. (2019). Effects of environmental enrichment on pig welfare—A review. *Animals*, 9(6), 383. <https://doi.org/10.3390/ani9060383>

Gonyou, H. W. (2001). The social behaviour of pigs. In *Social behaviour in farm animals* (pp. 147–176). CABI Publishing.

Goumon, S., Illmann, G., Leszkowová, I., Dostalová, A., & Cantor, M. (2020). Dyadic affiliative preferences in a stable group of domestic pigs. *Applied Animal Behaviour Science*, 230, 105045. <https://doi.org/10.1016/j.applanim.2020.105045>

Gustafsson, M., Jensen, P., de Jonge, F. H., & Schuurman, T. (1999). Domestication effects on foraging strategies in pigs (*Sus scrofa*). *Applied Animal Behaviour Science*, 62(4), 305–317. [https://doi.org/10.1016/s0168-1591\(98\)00236-6](https://doi.org/10.1016/s0168-1591(98)00236-6)

Jensen, P., & Wood-Gush, D. G. M. (1984). Social interactions in a group of free-ranging sows. *Applied Animal Behaviour Science*, 12(4), 327–337. [https://doi.org/10.1016/0168-1591\(84\)90125-4](https://doi.org/10.1016/0168-1591(84)90125-4)

Jowett, S., & Amory, J. (2021). The stability of social prominence and influence in a dynamic sow herd: A social network analysis approach. *Applied Animal Behaviour Science*, 238, 105320. <https://doi.org/10.1016/j.applanim.2021.105320>

Koenig, A. (2002). Competition for resources and its behavioral consequences among female primates. *International Journal of Primatology*, 23(4), 759–783. <https://doi.org/10.1023/a:1015524931226>

Kokocińska, A., & Kaleta, T. (2016). The role of ethology in animal welfare. *Scientific Annals of the Polish Society of Animal Production*, 12(1), 49–62. <https://doi.org/10.5604/01.3001.0013.6981>

Lagoda, M. E., Boyle, L. A., Marchewka, J., & Calderón Díaz, J. A. (2021). Mixing aggression intensity is associated with age at first service and floor type during gestation, with implications for sow reproductive performance. *Animal*, 15, 100158. <https://doi.org/10.1016/j.animal.2020.100158>

Lawrence, A. B., Newberry, R. C., & Špinka, M. (2018). Positive welfare. In *Advances in Pig Welfare* (pp. 415–444). Elsevier.

Mellor, D. J. (2015). Enhancing animal welfare by creating opportunities for positive affective engagement. *New Zealand Veterinary Journal*, 63(1), 3–8. <https://doi.org/10.1080/00480169.2014.926799>

Mellor, D. J., & Beausoleil, N. J. (2015). Extending the ‘Five Domains’ model for animal welfare assessment to incorporate positive welfare states. *Animal Welfare*, 24(3), 241–253. <https://doi.org/10.7120/09627286.24.3.241>

Moinard, C., Mendl, M., Nicol, C. J., & Green, L. E. (2003). A case control study of on-farm risk factors for tail biting in pigs. *Applied Animal Behaviour Science*, 81(4), 333–355. [https://doi.org/10.1016/s0168-1591\(02\)00276-9](https://doi.org/10.1016/s0168-1591(02)00276-9)

Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8. <https://doi.org/10.3389/fvets.2021.630164>

Norscia, I., Collarini, E., Robino, C., Chierto, E., & Cordoni, G. (2024). Witness for resolution: Post-conflict quadratic affiliation in semi-free ranging pigs. *Current Zoology*, 70, 233–243. <https://doi.org/10.1093/cz/zoad016>

Petersen, V., Simonsen, H. B., & Lawson, L. G. (1995). The effect of environmental stimulation on the development of behaviour in pigs. *Applied Animal Behaviour Science*, 45, 215–224. [https://doi.org/10.1016/0168-1591\(95\)00631-2](https://doi.org/10.1016/0168-1591(95)00631-2)

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jędrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLoS ONE*, 9, e99875. <https://doi.org/10.1371/journal.pone.0099875>

Seligman, M. E., & Csikszentmihalyi, M. (2000). Positive psychology: An introduction. *American Psychologist*, 55, 5–14. <https://doi.org/10.1037/0003-066X.55.1.5>

Siegel, S., & Castellan, N. J. J. (1988). *Nonparametric Statistics for the Behavioral Sciences*. McGraw Hill.

Simmins, P. H. (1993). Reproductive performance of sows entering stable and dynamic groups after mating. *Animal Science*, 57, 293–298. <https://doi.org/10.1017/s0003356100006917>

Špinka, M. (2017). Behaviour of pigs. In *The Ethology of Domestic Animals: An Introductory Text* (pp. 214–227). Wallington, UK: CABI.

Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Animal Science*, 48, 419–425. <https://doi.org/10.1017/s0003356100040411>

Studnitz, M., Jensen, K. H., & Jørgensen, E. (2003). The effect of nose rings on the exploratory behaviour of outdoor gilts exposed to different tests. *Applied Animal Behaviour Science*, 84, 41–57. [https://doi.org/10.1016/s0168-1591\(03\)00144-8](https://doi.org/10.1016/s0168-1591(03)00144-8)

Studnitz, M., Jensen, M. B., & Pedersen, L. J. (2007). Why do pigs root and in what will they root? *Applied Animal Behaviour Science*, 107, 183–197. <https://doi.org/10.1016/j.applanim.2006.11.013>

Temple, D., Manteca, X., Velarde, A., & Dalmau, A. (2011). Assessment of animal welfare through behavioural parameters in Iberian pigs in intensive and extensive conditions. *Applied Animal Behaviour Science*, 131, 29–39. <https://doi.org/10.1016/j.applanim.2011.01.013>

Van de Weerd, H. A., Docking, C. M., Day, J. E. L., Avery, P. J., & Edwards, S. A. (2003). A systematic approach towards developing environmental enrichment for pigs. *Applied Animal Behaviour Science*, 84, 101–118. [https://doi.org/10.1016/s0168-1591\(03\)00150-3](https://doi.org/10.1016/s0168-1591(03)00150-3)

Vanheukelom, V., Driessen, B., & Geers, R. (2012). The effects of environmental enrichment on the behaviour of suckling piglets and lactating sows: A review. *Livestock Science*, 143, 116–131. <https://doi.org/10.1016/j.livsci.2011.10.002>

General discussion

This thesis investigated various aspects of the social behaviour of domestic pigs and the management strategies for this species in naturalistic farming systems under extensive farming conditions through six studies. A key aim was to determine what socio-emotional behavioural and communication phenomena, possibly resulting from the domestication process started in the Neolithic period, may be present and used for welfare assessment and enhancement. In particular, by considering both biological and applied perspectives, this work has checked for the behavioural traits predicted by the “domestication syndrome,” and how these traits (leading to increased tolerance) can be leveraged to improve animal welfare in extensive farming contexts. In line with the aims of this thesis, my findings can contribute to a better understanding of these dynamics, offering insights into the potential implications for welfare management in pig farming.

In Chapters 1 and 2, it was found that the frequency of social play is generally higher in domestic pigs compared to wild boar hybrids. A significant difference was observed after the twentieth day of life, with domestic pigs engaging in social play more frequently than hybrids. However, play sessions in domestic pigs were shorter and less uniform than those of hybrids, indicating greater behavioural variability. Play fights were symmetrical and balanced in both groups. Contrary to expectations, aggression was more frequent in domestic pigs, with comparable levels during the first 35 days of life but higher than those of hybrids after the fifth week. Additionally, real fights in domestic pigs appeared more asymmetrical and predictable. Social play seems to contribute to the regulation of agonistic interactions, acting as a mechanism to reduce the risk of escalation in aggressive interactions. Social play appears to be primarily linked to motor training and the management of social interactions. Although domestic pigs played more frequently than wild boar hybrids, this behaviour did not translate into lower aggression levels or enhanced social stability. Social play, in this context, appears to serve functions related to motor training, social assessment, and the management of unexpected interactions. While play fighting shares some structural similarities with real fights, it remains distinct in terms of duration, symmetry, and variability, supporting the idea that it provides experience in handling competitive interactions. Rather than suggesting a direct role in social cohesion, these findings highlight a shift in behavioural expression that may reflect increased behavioural flexibility as a consequence of domestication. This distinction is essential for understanding the broader implications of behavioural adaptations in pigs, as it allows us to separate behaviours shaped by selection against aggression from those emerging as strategies to manage social dynamics.

The hypothesis that domestication has reduced aggression in pigs was only partially supported. While domestic pigs exhibited comparable aggression levels to wild boar hybrids in early life, their aggression became more frequent after the fifth week. Additionally, real fights in domestic pigs appeared more asymmetrical and predictable. Rather than simply selecting against aggression, domestication may have

favoured the emergence of alternative strategies for conflict management, allowing individuals to navigate social interactions more effectively. This suggests that the domestication process could have shaped conflict dynamics rather than merely reducing agonistic tendencies. These findings suggest that, rather than simply enhancing affiliative tendencies, domestication has favoured greater behavioural flexibility in play. Notably, social play does not appear to function primarily as a mechanism for reinforcing social bonds in domestic pigs. Instead, its structure suggests that it serves purposes such as motor training, social assessment, and the management of unexpected interactions. This is consistent with the fact that social play is not retained into adulthood, as it serves to develop these abilities during ontogeny. Chapters 1 and 2 provide key insights into how social interactions develop in domestic pigs, offering a basis for evaluating behavioural complexity in welfare assessments.

In Chapters 3 and 4, the focus was on post-conflict dynamics. Domestic pigs showed the ability to adopt complex strategies of reconciliation and triadic affiliation. Solicited triadic affiliations were predominantly initiated by the victim, particularly towards close kin. Conversely, unsolicited triadic affiliations were offered to both the victim and the aggressor by third-party individuals. Additionally, Chapter 4 revealed the presence of quadratic affiliations, where individuals not directly involved in the conflict established reciprocal interactions. These affiliations reduced anxiety in bystanders, thereby indirectly contributing to the maintenance of social homeostasis. Unlike social play, which does not show a clear function in social cohesion, reconciliation and third-party interventions actively contribute to group stability. Moreover, the presence of quadratic affiliations suggests that pigs can be influenced by social tensions even when not directly involved in conflicts, demonstrating elements of social appraisal and emotional regulation. This ability to engage in multi-level conflict resolution strategies could be a key behavioural adaptation shaped by domestication, as it allows individuals to manage the social complexities of group living more effectively. These findings further emphasize the importance of considering post-conflict behaviours when assessing welfare, as they provide measurable indicators of social stability and anxiety regulation. Indeed, my results could have direct implications for welfare management, demonstrating that specific interventions - such as optimizing social grouping and providing environmental enrichment - might enhance welfare while maintaining behavioural complexity. In particular, the behavioural insights gained from Chapters 1 to 4 could offer a framework for designing welfare strategies that align with pigs' natural social competencies.

In Chapters 5 and 6, practical applications were explored. The use of nose-clips proved effective in reducing destructive rooting without significantly impacting affiliative behaviours. The initial stress effects, measured through salivary cortisol levels and anxiety-related behaviours frequencies, were transient and returned to normal within three weeks of application. There was a temporary decrease in affiliative interactions involving the nose during the first week after the application of nose clips, but no differences in affiliative interactions without nose contact were observed. Furthermore,

the application of nose clips did not affect levels of aggressive behaviours and key social behaviours, such as post-conflict reconciliation and triadic affiliations, which are crucial for anxiety buffering and maintaining social stability. Group composition influenced social behaviours, with mixed-sex groups promoting higher levels of affiliation and more balanced social interactions. The use of enrichment materials, such as straw, reduced anxiety-related behaviours while simultaneously encouraging non-invasive exploratory activities. These enrichments also supported positive social interactions, including affiliative and post-conflict behaviours, which are essential for reducing stress and promoting overall welfare. Furthermore, the use of enrichment materials did not influence levels of aggressive behaviours. These findings have direct implications for welfare management, demonstrating that specific interventions—such as optimizing social grouping and providing environmental enrichment—can enhance welfare while maintaining behavioural complexity. In particular, the use of straw as an enrichment material reduced anxiety-related behaviours and encouraged non-invasive exploratory activities, reinforcing the idea that welfare-oriented strategies should aim to preserve natural behavioural repertoires rather than suppress them.

Overall, this thesis highlights that the domestication process may have not simply led to a generalized increase in affiliative behaviours or a reduction in aggression. Instead, it may have shaped a more flexible behavioural repertoire, where pigs retain the capacity for aggression but also exhibit sophisticated social regulation strategies. By linking fundamental research on social behaviour to applied welfare strategies, this work highlights how an understanding of domestication-driven behavioural modifications can inform more effective management practices. By understanding these dynamics, it is possible to develop more effective welfare interventions that align with the behavioural needs of domestic pigs, ensuring both social stability and environmental sustainability in extensive farming systems.

“Domesticated behaviour”: measures of pig socio-emotional behaviour

The domestication of pigs is thought to have led to significant changes in the behaviour and socio-emotional characteristics of this species. As highlighted in Chapters 1 and 2, one of the most relevant aspects is the role of social play in the development of motor and social skills in domestic pigs. Indeed, the findings of Chapters 1 and 2 indicate that social play is an essential component of the development of motor and social skills in domestic pigs. This domestication process has selected behavioural traits that include reduced aggression and increased social tolerance, often accompanied by physical changes known as the "domestication syndrome". Social play sessions showed progressive complexity over time, incorporating coordinated movements and signals that promote reciprocity, suggesting it provides a safe context for exploring and modulating social interactions. Social play

frequencies were significantly higher in domestic pigs compared to wild boar hybrids, particularly after the 20th day of life. This finding suggests that play, initially comparable between the two groups, becomes more prominent in domestic pigs during development, likely due to the neotenic nature of play behaviour—a characteristic associated with domestication that prolongs the playful phases of ontogeny (McKinney, 1998; Špinka et al., 2001; Palagi and Cordoni, 2012). Furthermore, play sessions in domestic pigs were characterised by greater reciprocity and symmetry compared to hybrids, reflecting a behavioural adaptation that promotes group cohesion and regulates agonistic interactions (Špinka et al., 2001; Cordoni et al., 2021). Finally, social play involves variability in motor patterns and less repetitiveness compared to aggression, elements that enable individuals to develop social and physical skills in a less conflictual context (Palagi et al., 2016; Pellis and Pellis, 2017). Regarding social play, pigs can exhibit coordinated movements and play signals that reduce the risk of conflict, enabling individuals to explore social dynamics in a safe context (McKinney, 1998; Palagi and Cordoni, 2012). This behaviour, common across many evolutionary lines in the animal kingdom, is particularly prevalent among certain birds and several mammals species (Burghardt, 2005). Complex play, which often includes simulated fights, has been proposed as a strategy to develop motor and socio-cognitive skills, providing long-term benefits (Smaldino, 2019). To maintain play as a non-conflictual activity, various species have evolved communicative signals that ensure fairness and mitigate the risk of escalation (Dugatkin and Bekoff, 2003; Palagi et al., 2016; Pellis and Pellis, 2017). Contrary to expectations associated with the domestication syndrome, aggression levels are higher in domestic pigs compared to wild boar hybrids. This suggests that artificial selection has had a greater impact on relationships between animals and humans than on interactions among conspecifics. This outcome could be attributed to the selective pressures in farming environments, where competition for limited resources in confined spaces promotes increased internal conflict (D'Eath and Turner, 2009; Wilkins et al., 2014).

This finding is crucial for the overall thesis as it highlights the complexity of domestication effects. While domestication may have led to increased docility towards humans, it may not have necessarily reduced aggression among conspecifics. This insight underscores the importance of considering environmental and social factors in welfare assessments and suggests that the behavioural dynamics in domestic pigs are influenced by the specific conditions of their environment.

These initial results are the basis for a more in-depth investigation of the social dynamics of domestic pigs, focusing in particular on the potential effects of the domestication process on behaviour. Recognizing that direct social interactions such as play and aggression are only part of the picture - also considering the significant decrease in social play levels observed after the first weeks of life of piglets -, I have shifted my focus to the post-conflict mechanisms that pigs employ to keep social tensions under control and restore homeostasis within groups. In Chapters 3 and 4, my studies highlight the ability of domestic pigs to adopt complex social strategies to

manage conflicts. In the post-conflict context, several affiliative modalities were documented, including reconciliation between former opponents and triadic and quadratic contacts involving individuals not directly involved in the conflict. In particular, consolation emerged as a crucial form of triadic affiliation, where a third individual interacts with one of the opponents, helping to reduce their anxiety and restore social homeostasis. These behaviours reflect an adaptation that promotes group cohesion and reduces collective anxiety (de Waal, 2000; Aureli et al., 1989). Moreover, these findings might suggest intrinsic and extrinsic regulation mechanisms as well as social appraisal processes (*sensu* Zaki and Williams 2013; Walle et al. 2017). The presence post-conflict affiliative interactions in pigs are consistent with what has been observed in other social mammal species (de Waal and van Roosmalen 1979; de Waal 2000; Aureli et al. 2002; Romero et al. 2009, 2011). Post-conflict social regulation through triadic affiliative contacts, both solicited and unsolicited, is a widespread phenomenon among social mammals, suggesting a common adaptive function in restoring social balance after an aggressive episode (Aureli et al. 2002; Aureli and Schaffner, 2002; Fraser and Aureli, 2008; McFarland and Majolo, 2012). Reconciliation occurs frequently in domestic pigs, often between individuals sharing weak social bonds, such as distant relatives. This finding contrasts with expectations suggesting that stronger bonds would necessitate more frequent reconciliation, indicating that pigs may strategically invest in weaker relationships to improve mutual tolerance in competitive social contexts. Such behaviour might reflect an evolutionary strategy aimed at maximising group cohesion, creating a more tolerant and stable social network (Andersen et al., 2004; Estevez et al., 2007). Moreover, observations of weaker bonds may underline the importance of flexible social dynamics, as described in other complex social species, such as primates (Cords and Aureli, 2000; Koski, 2015). Moreover, the involvement of third parties in supporting the victim or modulating the behaviour of the aggressor mirrors dynamics already documented in primates, such as in hamadryas baboons and Tonkean macaques, where triadic and quadratic post-conflict affiliations contribute to reducing collective anxiety and maintaining group cohesion (Judge and Mullen, 2005; De Marco et al., 2010; Daniel and Alves, 2015).

In social mammals, anxiety can be buffered through affiliative interactions, and this social buffering mechanism has been documented in both human and non-human primates (Seyfarth and Cheney, 2012; Aureli et al., 2000) and non-primate social mammals (Kikusui et al., 2006; Hennessy et al., 2009; Carter et al., 1998; Tamaki et al., 2006). Consistent with these findings, affiliative behaviours in pigs have been observed to significantly reduce anxiety levels (Reimert et al., 2014; Norscia et al., 2021). In particular, affiliative contacts play a key role in the post-conflict mechanisms of social mammals. Post-conflict behaviours in pigs, such as reconciliation and consolation, exhibit similarities with those observed in other social species. In primates, reconciliation often manifests through grooming or prolonged physical contact, which facilitates the restoration of social homeostasis and reduces post-conflict anxiety (de Waal, 2000; Aureli et al., 1989). Reconciliation has been shown to reduce the frequency of anxiety-related behaviours, particularly in victims of aggression, in social

mammalian species such as domestic goats (*Capra hircus*; Schino, 1998) and chimpanzees (*Pan troglodytes*; Kutsukake and Castle, 2001). Similarly, in gorillas (*Gorilla gorilla*), prolonged physical contact has been documented to promote reconciliation between opponents and stabilise group dynamics (Watts, 1995). Triadic contacts can effectively regulate the experience of former opponents by reducing self-anxiety levels, particularly in victims (Palagi and Cordoni, 2009; McFarland and Majolo, 2012; Puga-Gonzalez et al., 2014). More broadly, post-conflict affiliation can mitigate the anxiety induced by aggression in opponents and/or third parties involved in such affiliation across various social mammal species, including primates (de Waal and Aureli, 1997; Fraser et al., 2008, 2009). Moreover, the effects of aggression can reverberate throughout the group, increasing social tension, and quadratic affiliations, help reduce this tension (De Marco et al., 2010). In this regard, similarly to what I observed in pigs, hamadryas baboons (*Papio hamadryas*) display quadratic affiliative behaviours that reduce anxiety in group members following episodes of aggression (Judge and Mullen, 2005). Finally, in Tonkean macaques (*Macaca tonkeana*), both triadic and quadratic post-conflict affiliations have been associated with decreased collective anxiety (De Marco et al., 2010; Daniel and Alves, 2015).

Moreover, my studies have, for the first time, described the mechanism known as consolation in domestic pigs (Fujisawa et al., 2006; Fraser et al., 2008), a behaviour where a third party interacts with the victim of a conflict to alleviate their anxiety and restore social balance. This mechanism has also been documented for great apes and humans (chimpanzees: Fraser et al., 2008, Romero and de Waal, 2010; gorillas, Palagi et al., 2004; Cordoni and Palagi, 2007; bonobos, Clay and de Waal, 2013; Palagi and Norscia, 2013; humans, Fujisawa et al., 2006), highlighting deep similarities in social mechanisms between these species and domestic pigs. In primates, such as chimpanzees and bonobos, triadic contacts with the victim significantly contribute to the extrinsic regulation of anxiety (de Waal and van Roosmaleen, 1979; Cordoni et al., 2006; Romero and de Waal, 2010; Palagi and Norscia, 2013). Among bonobos (*Pan paniscus*), behaviours such as embracing and grooming hold strong consolatory value, effectively reducing the anxiety of individuals involved in conflicts (Clay and de Waal, 2013).

Focusing on social behaviours, my results suggest a potentially crucial role for emotional contagion. Specifically, my findings on post-conflict interactions could indicate that post-conflict affiliative behaviours occur in response to the distress of the conflict victim and other bystanders. In line with previous studies (Reimert et al. 2013, 2014, 2015; Norscia et al. 2021), the significant reduction in anxiety-related behaviours following these interactions suggests that pigs not only detect the emotional states of others but also respond in a way that modulates social tension. These results can confirm the hypothesis of emotional contagion in domestic pigs, as they imply a transmission of affective states that influences subsequent social dynamics. Indeed, emotional contagion, defined as the automatic sharing of emotional states between individuals, serves as a fundamental mechanism for social regulation in social species.

In domestic pigs, this phenomenon has been documented, with individuals responding to the distress of others through affiliative behaviours. These behaviours not only help reduce stress but also synchronise their emotions with those of other pigs, which respond emotionally in anticipation of future events (Reimert et al., 2013, 2014, 2015; Norscia et al., 2021). Such behavioural responses include physical contacts, vocalisations, and other interactions, reflecting an ability to perceive and respond to the emotional states of other group members. As highlighted by Norscia et al. (2021), emotional contagion in pigs aligns with observations in other social species from an evolutionary point of view, where the phenomenon has been interpreted as a precursor to empathy (Clay and de Waal, 2013; Tamaki et al., 2006). In this regard, it is important to take into account that consolation is a form of expressing empathy and is more complex than emotional contagion because it requires a greater ability to distinguish between self and other (Preston and de Waal, 2002). Moreover, recognising these aspects is essential for understanding the complexity of socio-emotional skills in domestic pigs and integrating them into welfare assessments.

In this context, the ability to share emotional states not only facilitates social regulation but also suggests an evolutionary link between phylogenetically distant species, indicating that emotional contagion may represent a fundamental adaptation for group living. According to Hall's (2012) definition, behavioural homologies can be recognised even when genetic or structural mechanisms are not shared, as they evolve in response to similar selective pressures. In this regard, my findings may highlight the importance of anxiety-buffering mechanisms in the stability and cohesion of social groups in domestic pigs. These findings also allow us to hypothesise that such behaviours might represent deep homologies among social mammalian species. The complex social behaviours observed in domestic pigs may serve as examples of potential deep homologies, connecting social play and post-conflict mechanisms to the shared evolutionary roots of other social mammalian species.

My results provide useful tools for an animal welfare assessment that considers a broader range of aspects compared to traditional approaches, in line with my aims. The documentation of post-conflict strategies - combined with previous findings on emotional contagion and anxiety-buffering mechanisms (Reimert et al., 2013, 2014, 2015; Norscia et al., 2021) - in domestic pigs enriches my understanding of the social dynamics of this species and provides valuable behavioural indicators for assessing their welfare. In particular, the ability of pigs to share emotional states and adopt affiliative behaviours in response to the distress of others and in conflict management mechanisms suggests that animal welfare should not be evaluated solely in terms of the absence of suffering but must also include the presence of positive experiences and the capacity for emotional regulation, in line with a modern view of animal welfare. Furthermore, the identification of deep behavioural homologies between domestic pigs and other social species, including primates, underscores the importance of considering the effects of domestication (or self-domestication) processes and the evolutionary roots of social behaviours in welfare assessment. This integrated

approach, combining ethological observations with physiological and environmental measures, allows for the development of management protocols that respect the behavioural needs of animals, thereby improving their overall welfare.

“Domesticated welfare”: evaluation of pig welfare based on behavioural, hormonal, and environmental measures

In the early chapters of this thesis, I explored the behavioural traits possibly resulting from domestication and focused on the role and ontogeny of social play and the role of affiliative behaviours in conflict management strategies. In particular, affiliative behaviours not only contribute to social cohesion but also play a crucial role in reducing anxiety and emotional regulation, and serve as a key tool in post-conflict mechanisms that help avoid the negative effects of aggression within the social group. Considering these observations, it is essential to consider how such social dynamics can be integrated into management practices to improve animal welfare. My results helped to develop welfare assessment methods that consider positive social experiences and the role that domestication may have played in shaping the social behaviour of pigs.

With regard to the management of domestic pigs in naturalistic farming contexts, considering the modern definition of animal welfare - which emphasises the importance of ensuring the presence of positive experiences in addition to minimising negative ones (Mellor, 2015; Mellor and Beausoleil, 2015; Špinka, 2017; Lawrence et al., 2018) - the findings of my studies on the social behaviour of domestic pigs provide valuable insights for a comprehensive evaluation of animal welfare. In farming contexts, positive social experiences, including those induced by emotional contagion and effective post-conflict mechanisms, are particularly relevant for assessing animal welfare as they highlight how positive social interactions can buffer the effects of stress and enhance the quality of life for animals. In this regard, Chapters 5 and 6 translate ethological observations into practical interventions, offering a significant contribution to improving animal welfare and sustainably managing natural habitats. These interventions are based on a deep understanding of the natural behaviours and needs of animals, emerging from the preceding chapters and studies that have documented phenomena such as anxiety-buffering mechanisms and emotional contagion in domestic pigs (Reimert et al., 2013, 2014, 2015; Norscia et al., 2021).

An example is the use of nose clips in domestic pigs. Nose-clipping could represent a valid alternative to the application of nose rings, which restrict rooting but may cause chronic discomfort. My results have shown that nose clips can be considered a minimally invasive alternative, providing an effective compromise between management and welfare. Despite a slight initial increase in stress, as measured by cortisol levels, affiliative behaviours were not negatively affected. It is crucial to consider that domestic pigs have an inherent need for exploratory behaviours and use their snouts to investigate their environment and objects. Limiting this behaviour could

lead to a degradation in welfare, with consequent increases in stress levels (Studnitz et al., 2007). On the other hand, positive social interactions, including affiliative behaviours, can play a crucial role in promoting and enhancing animal health and welfare (Rault, 2012; Rault, 2019). Ideally, pigs should not be prevented from rooting to maximise their welfare. However, the findings indicate that the application of nose clips could be a minimally invasive and effective technique for preserving the natural environment without disrupting positive social dynamics. This is because pigs' responses to the application of nose clips appear to trigger a series of events aimed at coping with stress conditions and restoring homeostasis at both individual and group levels. In this context, affiliative behaviours and post-conflict management mechanisms play a fundamental role as positive experiences that help regulate aspects related to negative experiences, such as the inability to root, the application of nose clips, or intra-group aggression. This approach may offer a solution, adequately balancing animal welfare needs with environmental concerns. Therefore, the results suggest that allowing domestic pigs to express their behavioural repertoire - restricting only rooting behaviour while simultaneously enabling them to engage in positive social interactions - could help to keep anxiety levels under control, ensuring high animal welfare standards.

Due to the unexpected emergence and spread of African swine fever in north-eastern Italy, the work in Chapter 6 should be considered preliminary as I was unable to develop it as intended because pigs had to be moved to indoor facilities. Other possible applications could not be investigated as planned. However, the results obtained are encouraging and could serve as a basis for developing a more detailed research idea in the future, which also takes into account ecological parameters to assess the impact of free-ranging pigs on the vegetation cover characterising the woodland habitat where the animals were raised. Group composition has emerged as another important factor to consider in the management of domestic pig groups raised in semi-free-range conditions. While mixed-sex groups, composed of females and castrated males, displayed higher levels of invasive rooting behaviour, they also exhibited significantly higher levels of affiliative behaviours and post-conflict interactions compared to single-sex groups of either males or females. These behaviours help reduce anxiety in the individuals involved (Norscia et al., 2021) and, as described in Chapters 3 and 4, include consolatory contacts and reconciliations that can enhance social cohesion. In this context, the higher frequency of post-conflict affiliations in mixed-sex groups may reflect a compensatory strategy to address the potential social instability linked to their artificial composition. Mixed-sex groups, created artificially, are likely to be less stable socially, unlike natural groupings consisting of related females and young males, which tend to exhibit a stable composition (Stolba and Wood-Gush, 1989; Gabor et al., 1999; D'Eath and Turner, 2008; Gonyou, 2001). Social cohesion in mixed groups may stem from effective hierarchy management through limited and swiftly resolved agonistic interactions facilitated by post-conflict strategies, thereby preventing stress escalation (De Waal, 2000; Gonyou, 2001). Environmental enrichment is another important aspect for the

management of domestic pig groups raised in extensive systems, with implications for animal welfare. The application of management strategies, such as introducing enrichment materials - particularly manipulable items like straw - has been shown to significantly reduce anxiety-related behaviours and promote non-invasive exploratory activities. This type of enrichment not only meets the pigs' intrinsic behavioural needs but also reduces the prevalence of anxiety-driven and aggressive behaviours, contributing to improvements in both the psychological and physical welfare of the animals (van de Weerd et al., 2009; Day et al., 2001; Beattie et al., 2000). In my study, straw was particularly effective in balancing the need to minimise destructive rooting while maintaining positive social dynamics within the group.

Therefore, implementing protocols that consider social dynamics - such as fostering affiliative interactions and maintaining stable hierarchies - can help enhance group stability and reduce overall stress levels (Špinka et al., 2017). In particular, the formation of mixed-sex groups, the provision of environmental enrichments, and the application of nose clips may represent effective measures for mitigating invasive digging behaviours while simultaneously providing opportunities for social interactions that alleviate stress and maintain social cohesion. The insights gained from this research provide valuable guidance for developing management protocols that balance animal welfare with environmental sustainability. For example, the use of nose clips in combination with environmental enrichment and the management of group composition could represent a practical and effective solution to reduce the negative impact of pigs on natural habitats without compromising their health and welfare.

Conclusions and future perspectives

In line with the aims of this thesis, my findings provide evidence that domestic pigs exhibit complex social behaviours, particularly in conflict management and emotional regulation, possibly shaped by domestication. In this sense, my work could serve as a basis for further research to clarify the extent of this influence and aspects related to selection against aggression. Despite the challenges posed by the spread of African swine fever, I successfully applied an ethological approach to assess welfare in an extensive farming context, integrating behavioural and hormonal measurements to gain a more comprehensive understanding of social regulation mechanisms. Additionally, I considered aspects related to an anthropological perspective, which allowed us to better understand the effects of the domestication process on behaviour.

My approach highlighted how integrating social dynamics into management protocols can not only enhance the assessment of animal welfare but also provide new perspectives on the environmental sustainability of farming systems. In this regard, such interventions may not be limited to domestic pigs but could also be extended to other domestic species that share social behavioural traits with pigs, opening new avenues for sustainable livestock management.

This research offers several innovative contributions. First, I analysed for the first time the structural differences between play and aggression, distinguishing their key characteristics and examining how these behaviours manifest in piglets compared to wild-boar hybrids to gain insight into how the domestication process may have shaped such social behaviours. Second, I conducted the first structured investigation into post-conflict strategies in domestic pigs, demonstrating the presence of reconciliation, triadic, and quadratic affiliations. In this sense, my studies have systematically described these behaviours, providing new insights into their social function and aspects that may be related to selection against aggression, as observed in other social species. Finally, by combining behavioural and hormonal data, I developed an integrated approach that enhances the reliability of welfare assessments, integrating behavioural indicators and physiological stress responses. Therefore, I believe that an ethological approach - adopting a bio-anthropological perspective - represents an innovative complement to agro-veterinary, clinical, and comparative psychology traditional approaches, providing a broader framework for understanding social regulation in domestic animals. In this regards, future research could integrate additional physiological parameters commonly used in animal welfare studies to further support the ethological perspective.

In sum, the findings of this thesis reveal the behavioural and social complexity of domestic pigs, highlighting their potential as a model species for studying conflict management and emotional regulation in socially complex mammals. The similarities between the social behaviours of domestic pigs and those of other group-living species, including humans, suggest that these traits may result from both evolutionary convergence and shared socio-ecological pressures. Moreover, while the domestication process may have reinforced certain aspects related to social tolerance and selection against aggression, further comparative studies are needed to determine the specific evolutionary mechanisms underlying these behaviours.

From a management perspective, it is important to acknowledge that attempting to eliminate all stressors could lead to a reduction in both environmental stimulation and social support exchanges, which are key elements for regulating anxiety levels and maintaining the physical and psychological health of animals (Špinka, 2006; Ozbay et al., 2007; Rault, 2012). In this regard, the introduction of species-specific enrichment materials, combined with the assessment of social relationships and environmental contexts, can help improve animal welfare across different production systems, in line with the modern understanding of animal welfare (Mellor, 2015, 2016; Mellor and Beausoleil, 2015; Fraser, 2008).

In conclusion, this research shows how applied ethology and aspects related to an anthropological approach can be translated into innovative management strategies that address both the behavioural and social needs of domestic pigs while simultaneously promoting sustainable farming practices. By recognising the complexity of pig social behaviour, possibly shaped by the process of domestication started in the Neolithic, and incorporating it into welfare assessments. My findings

emphasise the importance of an ethological perspective in developing management strategies that align animal welfare with environmental sustainability.

References

- Andersen, L. B., Hasselstrøm, H., Grønfeldt, V., Hansen, S. E., & Karsten, F. (2004). The relationship between physical fitness and clustered risk, and tracking of clustered risk from adolescence to young adulthood: eight years follow-up in the Danish Youth and Sport Study. *The International Journal of Behavioral Nutrition and Physical Activity*, 1(1). doi:10.1186/1479-5868-1-6
- Aureli, F. (2001). *Natural Conflict Resolution* (F. Aureli, Ed.). University of California Press. <https://doi.org/10.1525/9780520924932>
- Aureli, F., Cords, M., & van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: a predictive framework. *Animal Behaviour*, 64(3), 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Aureli, F., & Schaffner, C. M. (2002). Relationship assessment through emotional mediation. *Behaviour*, 139(2–3), 393–420. <https://doi.org/10.1163/156853902760102726>
- Aureli, F., Van Schaik, C. P., & Hooff, V. (1989). Functional aspects of reconciliation among captive long tailed macaques (*Macaca fascicularis*). *American Journal of Primatology*, 19(1), 39–51.
- Beattie, V. E., O'Connell, N. E., & Moss, B. W. (2000). Influence of environmental enrichment on the behaviour, performance and meat quality of domestic pigs. *Livestock Production Science*, 65(1–2), 71–79. [https://doi.org/10.1016/s0301-6226\(99\)00179-7](https://doi.org/10.1016/s0301-6226(99)00179-7)
- Burghardt, G. M. (2005). *The Genesis of Animal Play: Testing the limits*. The MIT Press. <https://doi.org/10.7551/mitpress/3229.001.0001>
- Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*, 23(8), 779–818. [https://doi.org/10.1016/s0306-4530\(98\)00055-9](https://doi.org/10.1016/s0306-4530(98)00055-9)
- Clay, Z., & de Waal, F. B. M. (2013). Bonobos respond to distress in others: consolation across the age spectrum. *PloS One*, 8(1), e55206. <https://doi.org/10.1371/journal.pone.0055206>
- Cordoni, G., Gioia, M., Demuru, E., & Norscia, I. (2021). The dark side of play: play fighting as a substitute for real fighting in domestic pigs, *Sus scrofa*. *Animal Behaviour*, 175, 21–31. <https://doi.org/10.1016/j.anbehav.2021.02.016>
- Cordoni, G., Palagi, E., & Tarli, S. B. (2006). Reconciliation and consolation in captive western gorillas. *International Journal of Primatology*, 27(5), 1365–1382. <https://doi.org/10.1007/s10764-006-9078-4>
- Cordoni, G., & Palagi, E. (2007). Response of captive lowland gorillas (*Gorilla gorilla gorilla*) to different housing conditions: testing the aggression-density and coping models. *Journal of Comparative Psychology (Washington, D.C.: 1983)*, 121(2), 171–180. <https://doi.org/10.1037/0735-7036.121.2.171>
- Cords, M., & Aureli, F. (2000). Reconciliation and relationship qualities. In F. B. M. Aureli F (Ed.), *Natural conflict resolution* (pp. 177–198). University of California Press.

Daniel, J. R., & Alves, R. L. (2015). Postconflict affiliation among bystanders in a captive group of Japanese macaques (*Macaca fuscata*). *International Journal of Primatology*, 36(2), 259–268. <https://doi.org/10.1007/s10764-015-9822-8>

Day, J. E. L., Spooler, H. A. M., & Edwards, S. A. (2001). Straw as environmental enrichment: Which properties do growing pigs find behaviourally rewarding? In *Proceedings of the International Symposium of the C.I.G.R. Animal Welfare Considerations in Livestock Housing Systems* (pp. 157–167).

De Marco, M., Cozzolino, A., Dessì-Fulgheri, R., & Thierry, F. (2010). Conflicts induce affiliative interactions among bystanders in a tolerant species of macaque (*Macaca tonkeana*). *Animal Behaviour*, 80(2), 197–203.

de Waal, F. B. (2000). Primates—a natural heritage of conflict resolution. *Science (New York, N.Y.)*, 289(5479), 586–590. <https://doi.org/10.1126/science.289.5479.586>

de Waal, F., & Aureli, F. (1997). Conflict resolution and distress alleviation in monkeys and apes. In C. S. Carter (Ed.), *The integrative neurobiology of affiliation. Lederhendler II, Kirkpatrick B* (pp. 317–328). New York Academy of Sciences.

de Waal, F. B. M., & van Roosmalen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioral Ecology and Sociobiology*, 5(1), 55–66. <https://doi.org/10.1007/bf00302695>

D'eath, R. B., & Turner, S. P. (2009). The natural behaviour of the pig. In J. N. Marchant-Forde (Ed.), *The welfare of pigs* (pp. 13–45). Springer.

Dugatkin, L. A., & Bekoff, M. (2003). Play and the evolution of fairness: a game theory model. *Behavioural Processes*, 60(3), 209–214. [https://doi.org/10.1016/s0376-6357\(02\)00120-1](https://doi.org/10.1016/s0376-6357(02)00120-1)

Estevez, I., Andersen, I. L., & Nævdal, E. (2007). Group size, density and social dynamics in farm animals. *Applied Animal Behaviour Science*, 103(3–4), 185–204. <https://doi.org/10.1016/j.applanim.2006.05.025>

Fraser, D. (2008). *Understanding animal welfare: The science in its cultural context*. Wiley-Blackwell.

Fraser, O. N., & Aureli, F. (2008). Reconciliation, consolation and postconflict behavioral specificity in chimpanzees. *American Journal of Primatology*, 70, 1–10. <https://doi.org/10.1002/ajp.20608>

Fraser, O. N., Koski, S. E., Wittig, R. M., & Aureli, F. (2009). Why are bystanders friendly to recipients of aggression? *Communicative & Integrative Biology*, 2(3), 285–291. doi:10.4161/cib.2.3.8718

Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*, 105(25), 8557–8562. <https://doi.org/10.1073/pnas.0804141105>

Fujisawa, K. K., Kutsukake, N., & Hasegawa, T. (2006). Peacemaking and consolation in Japanese preschoolers witnessing peer aggression. *Journal of Comparative Psychology (Washington, D.C.: 1983)*, 120(1), 48–57. <https://doi.org/10.1037/0735-7036.120.1.48>

Gonyou, H. W. (2001). The social behaviour of pigs. In *Social behaviour in farm animals* (pp. 147–176). CABI Publishing.

Hall, B. K. (2013). Homology, homoplasy, novelty, and behavior. *Developmental Psychobiology*, 55(1), 4–12. <https://doi.org/10.1002/dev.21039>

Hennessy, M. B., Kaiser, S., & Sachser, N. (2009). Social buffering of the stress response: diversity, mechanisms, and functions. *Frontiers in Neuroendocrinology*, 30(4), 470–482. <https://doi.org/10.1016/j.yfrne.2009.06.001>

Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, 69(6), 1345–1355. <https://doi.org/10.1016/j.anbehav.2004.08.016>

Kikusui, T., Winslow, J. T., & Mori, Y. (2006). Social buffering: relief from stress and anxiety. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 361(1476), 2215–2228. <https://doi.org/10.1098/rstb.2006.1941>

Koski, S. E. (2015). Reconciliation and peace-making: Insights from studies on nonhuman animals. In *Emerging Trends in the Social and Behavioral Sciences* (pp. 1–9). Wiley. <https://doi.org/10.1002/9781118900772.etrds0276>

Kutsukake, N., & Castles, D. L. (2004). Reconciliation and post-conflict third-party affiliation among wild chimpanzees in the Mahale Mountains, Tanzania. *Primates*, 45, 157–165. <https://doi.org/10.1007/s10329-004-0082-z>

McFarland, R., & Majolo, B. (2012). The occurrence and benefits of postconflict bystander affiliation in wild Barbary macaques (*Macaca sylvanus*). *Animal Behaviour*, 84(3), 583–591. <https://doi.org/10.1016/j.anbehav.2012.06.010>

Mckinney, M. L. (1998). *Evolution, and Development; Psychology Press*. Psychology Press.

Mellor, D. J. (2015). Enhancing animal welfare by creating opportunities for positive affective engagement. *New Zealand Veterinary Journal*, 63(1), 3–8. <https://doi.org/10.1080/00480169.2014.926799>

Mellor, D. J., & Beausoleil, N. J. (2015). Extending the ‘Five Domains’ model for animal welfare assessment to incorporate positive welfare states. *Animal Welfare (South Mimms, England)*, 24(3), 241–253. <https://doi.org/10.7120/09627286.24.3.241>

Mellor, D. J. (2016). Updating animal welfare thinking: Moving beyond the “Five Freedoms” towards “A life worth living.” *Animals: An Open Access Journal from MDPI*, 6(3), 21. <https://doi.org/10.3390/ani6030021>

Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>

Ozbay, F., Johnson, D. C., Dimoulas, E., Morgan, C. A., Charney, D., & Southwick, S. (2007). Social support and resilience to stress: From neurobiology to clinical practice. *Psychiatry*, 4(5), 35–40.

Palagi, E., Burghardt, G. M., Smuts, B., Cordoni, G., Dall’Olio, S., Fouts, H. N., Řeháková-Petrů, M., Siviý, S. M., & Pellis, S. M. (2016). Rough-and-tumble play as a window on animal

communication: Social play and communication in animals. *Biological Reviews of the Cambridge Philosophical Society*, 91(2), 311–327. <https://doi.org/10.1111/brv.12172>

Palagi, E., & Cordoni, G. (2009). Postconflict third-party affiliation in *Canis lupus*: do wolves share similarities with the great apes? *Animal Behaviour*, 78(4), 979–986. <https://doi.org/10.1016/j.anbehav.2009.07.017>

Palagi, E., & Cordoni, G. (2012). The right time to happen: play developmental divergence in the two *Pan* species. *PloS One*, 7(12), e52767. <https://doi.org/10.1371/journal.pone.0052767>

Palagi, E., & Norscia, I. (2013). Bonobos protect and console friends and kin. *PloS One*, 8(11), e79290. <https://doi.org/10.1371/journal.pone.0079290>

Palagi, E., Paoli, T., & Tarli, S. B. (2004). Reconciliation and consolation in captive bonobos (*Pan paniscus*). *American Journal of Primatology*, 62(1), 15–30. <https://doi.org/10.1002/ajp.20000>

Pellis, S. M., & Pellis, V. C. (2017). What is play fighting and what is it good for? *Learning & Behavior*, 45(4), 355–366. <https://doi.org/10.3758/s13420-017-0264-3>

Puga-Gonzalez, I., Butovskaya, M., Thierry, B., & Hemelrijk, C. K. (2014). Empathy versus parsimony in understanding post-conflict affiliation in monkeys: Model and empirical data. *PLOS ONE*, 9(3), e91262. <https://doi.org/10.1371/journal.pone.0091262>

Rault, J.-L. (2012). Friends with benefits: Social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science*, 136(1), 1–14. <https://doi.org/10.1016/j.applanim.2011.10.002>

Rault, J.-L. (2019). Be kind to others: Prosocial behaviours and their implications for animal welfare. *Applied Animal Behaviour Science*, 210, 113–123. <https://doi.org/10.1016/j.applanim.2018.10.015>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109, 42–50. <https://doi.org/10.1016/j.physbeh.2012.11.002>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology & Behavior*, 129, 221–229. <https://doi.org/10.1016/j.physbeh.2014.02.059>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, 18(2), 517–532. <https://doi.org/10.1007/s10071-014-0820-6>

Romero, T., Castellanos, M. A., & de Waal, F. B. M. (2011). Post-conflict affiliation by chimpanzees with aggressors: other-oriented versus selfish political strategy. *PloS One*, 6(7), e22173. doi:10.1371/journal.pone.0022173

Romero, T., Colmenares, F., & Aureli, F. (2009). Testing the function of reconciliation and third-party affiliation for aggressors in hamadryas baboons (*Papio hamadryas hamadryas*). *American Journal of Primatology*, 71(1), 60–69. doi:10.1002/ajp.20619

- Romero, T., & de Waal, F. B. M. (2010). Chimpanzee (*Pan troglodytes*) consolation: third-party identity as a window on possible function. *Journal of Comparative Psychology (Washington, D.C.: 1983)*, 124(3), 278–286. <https://doi.org/10.1037/a0019144>
- Schino, G. (1998). Reconciliation in domestic goats. *Behaviour*, 135(3), 343–356. <https://doi.org/10.1163/156853998793066302>
- Seyfarth, R. M., & Cheney, D. L. (2012). The evolutionary origins of friendship. *Annual Review of Psychology*, 63(1), 153–177. <https://doi.org/10.1146/annurev-psych-120710-100337>
- Smaldino, P. E. (2019). The evolution of play: A formal model based on costs and benefits. *Behavioral Ecology and Sociobiology*, 73.
- Špinka, M. (2017). Behaviour of pigs. In *The ethology of domestic animals: an introductory text* (pp. 214–227). CABI.
- Spinka, M., Newberry, R. C., & Bekoff, M. (2001). Mammalian play: training for the unexpected. *The Quarterly Review of Biology*, 76(2), 141–168. <https://doi.org/10.1086/393866>
- Studnitz, M., Jensen, M. B., & Pedersen, L. J. (2007). Why do pigs root and in what will they root? *Applied Animal Behaviour Science*, 107(3–4), 183–197. <https://doi.org/10.1016/j.applanim.2006.11.013>
- Tamaki, N. (2006). Social bonding and affiliative interactions in dolphins: Post-conflict reconciliation. *Marine Mammal Science*, 22(3), 843–860.
- Van de Weerd, H. A., Docking, C. M., Day, J. E. L., Avery, P. J., & Edwards, S. A. (2003). A systematic approach towards developing environmental enrichment for pigs. *Applied Animal Behaviour Science*, 84(2), 101–118. [https://doi.org/10.1016/s0168-1591\(03\)00150-3](https://doi.org/10.1016/s0168-1591(03)00150-3)
- Watts, D. P. (1995). Post-conflict social events in wild mountain gorillas (mammalia, hominoidea). I. social interactions between opponents. *Ethology: Formerly Zeitschrift Für Tierpsychologie*, 100(2), 139–157. <https://doi.org/10.1111/j.1439-0310.1995.tb00321.x>
- Walle, E., Reschke, P., Camras, L., & Campos, J. (2017). Infant differential behavioral responding to discrete emotions. *Emotion*, 17, 1078–1091. <https://doi.org/10.1037/emo0000307>
- Wilkins, A. S., Wrangham, R. W., & Fitch, W. T. (2014). The “domestication syndrome” in mammals: a unified explanation based on neural crest cell behavior and genetics. *Genetics*, 197(3), 795–808. <https://doi.org/10.1534/genetics.114.165423>
- Zaki, J., & Williams, W. C. (2013). Interpersonal emotion regulation. *Emotion*, 13(5), 803–810. <https://doi.org/10.1037/a0033839>

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Appendix

Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164.

A1. Anxiety Behavior in Pigs (*Sus scrofa*) Decreases Through Affiliation and May Anticipate Threat

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Abstract

Anxiety is a physio-psychological state anticipating an imminent threat. In social mammals it is behaviorally expressed *via* displacement activities and buffered *via* affiliation. Anxiety research on domestic pigs (*Sus scrofa*) has mostly focused on abnormal/stereotypic behavior associated with intensive farming. We investigated how anxiety is expressed and modulated in semi-free ranging pigs, in natural habitats. Owing to pigs' socio-cognitive complexity, we posited that displacement activities, if such, would increase after a (stressful) intra-group aggression (Prediction 1), be reduced by affiliation (Prediction 2) and influenced by individual/contextual factors (Prediction 3). From 224 videos recorded on adult individuals (Mean $\pm$ SD/subject: 4.84 $\pm$ 1.85 h) at the “Ethical Farm Parva Domus” (Turin, Italy), we extracted possible displacement activities (*vacuum-chewing*, *scratching/body-rubbing*, *head/body-shaking*, and *yawning*) in four 3-min conditions: before (BA) and after aggression events, in the absence (AA) or presence (AP) of post-aggression affiliation, and a matched-control (no event; MC). We conducted a minute-by-minute analysis in AE/AA and assessed the effect of subjects' involvement in a conflict (aggressor, aggression's recipient, bystander). All activities were higher in AA than in BA condition—thus being anxiety markers—and all of them decreased to baseline levels in AP, faster compared to AE. Hence, anxiety behavior in pigs was socially buffered. Intriguingly, anxiety behavior was expressed significantly more by bystanders than opponents, which suggests that pigs may be able to anticipate imminent threats. By highlighting how anxiety is managed under extensive farming, this study contributes to the understanding of pig welfare and biology.

A1.1 Introduction

In its broader definition, anxiety is an affective state that in both humans and other mammals is characterized by tension and/or agitation and is often behaviorally expressed in association with a physiological stress response (Van Riezen and Segal, 1988; Craig et al., 1995; Barros and Tomaz, 2002; Troisi, 2002; Bourin et al., 2007). In mammals, including *Homo sapiens*, genetic predisposition, early life experiences, and overcrowding conditions can increase the propensity to develop pathological anxiety and/or anxiety-related stereotypic behavior (Jeffcoate et al., 1986; Gordon and Hen, 2004; Latham and Mason, 2008; Ferdowsian et al., 2011; Tiira and Lohi, 2015; Martínez-Miró et al., 2016; McCoy et al., 2017; Malik et al., 2019; Salonen et al., 2020). However, non-pathological anxiety is commonly present in a mammal's life as a psychological, physiological, and behavioral response that helps the subjects to deal with unexpected or challenging situations (Steimer, 2002). Probably owing to this basic function, convergence in some anxiety related behaviors has been observed between humans and non-human mammals (Blanchard et al., 2001).

Intra-group aggression is a major source of anxiety in social mammals (Aureli et al., 2002). Indeed, agonistic encounters carry the risk of physical harm for individuals and can be disruptive for social groups, unless they are resolved via conflict management (de Waal, 2000). The anxiety response to aggression can not only be present but may also vary depending on the role played by individuals in the conflict [e.g., pigs (Muráni et al., 2010); rats, *Rattus norvegicus* (Neumann et al., 2010); various non-human primate species (Cords and Thurnheer, 1993; Aureli et al., 2002; Fraser et al., 2008; Palagi and Norscia, 2010; Crofoot, 2013; Norscia and Palagi, 2016)]. For example, the aggressor can show lower levels of anxiety than the aggression's recipient and the bystanders not involved in the conflict can also show an increase in anxiety [e.g., rats (Veenema and Neumann, 2007); *Lemur catta* (Sclafani et al., 2012); *Papio anubis* (Virgin and Sapolsky, 1997); *Papio hamadryas* (Judge and Mullen, 2005; Judge and Bachmann, 2013)]. The behavioral expression of anxiety in agonistic contexts is therefore necessary to ensure effective communication between individuals within a society and allow the enactment of social buffering measures (Neumann et al., 2010; de Waal and Preston, 2017; Kikusui et al., 2006).

Besides the behaviors that in animals are generally associated with the stress response to fear [e.g., urinating, defecating, escape (Davis, 1997)], there are other displacement activities more strictly linked to anxiety. Some are present in a variety of mammalian species and are therefore interesting to investigate in the light of future comparative studies. For example, depending on the severity of the stressor eliciting a response, self-scratching (hereafter scratching), yawning, vacuum behavior [sensu (Tinbergen, 1952)], and head/body shaking or trembling, can be associated with chronic and/or acute anxiety in both human and non-human primates (Maestriperi et al., 1992; Fried, 1994; Baker and Aureli, 1997; Bögels and Reith, 1999; Troisi, 2002; Guggisberg et al., 2010) and in other mammals, including domestic animals (Beerda et al., 2000; Kaiser et al., 2006; Lesimple et al., 2012; Cordoni and Norscia, 2014; Palagi et al., 2019). From the physiological perspective, such behaviors appear to be

linked to the hormonal cascade that underlies the stress response, mediated by cortisol and whose intensity depends on the stressor (Beerda et al., 2000; Thompson, 2011; Kubota et al., 2014; Zhang et al., 2017). Scratching, in particular, is performed in response to the itch sensation mediated by cortisol (Hachisuka et al., 2018; Sanders and Akiyama, 2018) and can lead to the itch-scratch cycle (Rinaldi, 2019).

In social mammals, anxiety can be buffered by affiliative interactions between subjects, such as closeness, body contact, and allogrooming (Kikusui et al., 2006). This social buffering mechanism has been observed in both primates [humans (Thorsteinsson et al., 1998); non-human primates (van Wolkenten et al., 2006; Aureli and Yates, 2010)], and non-primate mammals [rats (Davitz and Mason, 1955); guinea pigs, *Cavia porcellus* (Hennessy et al., 2000); prairie voles, *Microtus ochrogaster* (Burkett et al., 2016); pigs (Reimert et al., 2014)]. Indeed, affiliative contacts are known to reduce anxiety behavior in rodents (Beery and Kaufer, 2015), even though primates have been the most investigated group in this respect (Norscia and Palagi, 2016). From strepsirrhines to apes, displacement behavior has been found to be reduced after grooming (Sclafani et al., 2012), play (Norscia and Palagi, 2011), and post-conflict affiliative contacts with a former opponent or another group mate [(Aureli and van Schaik, 1991; Castles and Whiten, 1998; Das et al., 1998; Fraser et al., 2010); but see (Koski et al., 2007)]. Studies of diverse mammalian species, including humans, suggest that affiliation may cause the reduction of stress related anxiety via the activation of oxytocin, progesterone and/or endorphines mediated responses (Keverne et al., 1989; Brown et al., 2009; Smith and Wang, 2014). In an experimental setting, domestic pigs have been found to prefer staying close to familiar rather than unfamiliar subjects after a stressful conflict (Camerlink et al., 2014).

Although commonly known and available as study species, the domestic pig (*Sus scrofa*) has received relatively little attention with respect to its social behavior in extensive, naturalistic farming conditions because the majority of clinical ethology research has focused on behavioral alterations related to intensive, industrial food production chain. More specifically, stress and anxiety in the domestic pig *Sus scrofa* have been broadly investigated with respect to intensive farming [for a review (Martínez-Miró et al., 2016)]. However, to our knowledge no ethological study has so far quantitatively demonstrated the association of certain behaviors of *Sus scrofa* with the non-stereotypic display of anxiety and social buffering under naturalistic conditions. Yet, the domestic pig, *Sus scrofa*, is an excellent species to investigate emotional expression and modulation potentials in non-human social mammals, owing to its complex sociality, cognition and psychology (Marino and Colvin, 2015). Moreover, it seems that domestic species display more anxiety-like and less risk-taking and exploratory behavior than wild forms, as a result of the domestication process (Kaiser et al., 2015).

The aim of this study was to investigate the behavioral manifestation of transient anxiety and its management in *Sus scrofa*, by adopting the same approach used for non-domestic species, in an ecologically sound context. In this condition—as it also occurs in other settings (e.g., open field tests)—the subjects can express their

behavioral repertoire without the distortion caused by confinement and overcrowding. This issue is relevant not only to the understanding of pig emotional expression and modulation, but also to gain insights into pig welfare, considering the growing interest toward extensive and environmentally sustainable farming. To achieve this aim, we examined a suite of behaviors associated with chronic conditions in pigs or to chronic/acute stress in other mammals, and checked whether they could also be connected to transient anxiety in pigs. In particular, we measured the fluctuation of these behaviors in semi-free ranging pigs, living in a 13 ha habitat of natural mixed wood and grassland.

Based on the framework presented above, we expected that in *Sus scrofa*: (i) body shaking, vacuum chewing, yawning, and the behavior associated with the itch-scratch cycle (scratching/rubbing)—if linked to non-pathological anxiety—would increase after an aggressive event (Prediction 1); (ii) such behavior—if socially buffered—would be reduced in the subjects after affiliation with group mates (Prediction 2); (iii) the occurrence of anxiety related behavior—if modulated according to the risk faced by the subjects—could vary according to the role the subjects played in the aggression (i.e., aggressor, recipient or bystander) (Prediction 3).

A1.2 Materials and Methods

A1.2.1. Study Group and Site

This study was conducted from June to November 2018 on a group of free-ranging domestic pigs (*Sus scrofa*) at the Ethical Farm “Parva Domus” located at Cavagnolo, Turin (Italy). The animals could freely move and forage in a natural habitat, including both grassland and woodland, within a fenced area of 13 ha. The 104 adult subjects (7–22 months old) lived in the same group and included 54 males and 50 females of three mixed breeds: Parma Black, Landrace, and Piedmont Black. The animals had been together from 3 to 14 months and no subject showed stereotypic behavior during the study (e.g., repeated and abnormal behaviors in absence of any perturbing event). Owing to controlled reproduction, kinship varied from second cousins to full siblings. No parental relationship was present in the group. The males were castrated *via* the removal of testes within their first days of life, whereas females were potentially reproductive but reproductive males was kept in separate enclosures. Four feeding spots were available in the area where the animals were provided with food (Ciclo Unico P, SILDAMIN®) every day from 8:30 to 10:30 and water was available *ad libitum*. The subjects could supplement their food intake with roots, leaves or fruits naturally available in the environment. In order to allow the individual recognition of all subjects, the pigs were marked with spray Raidex® for livestock. Each individual had a unique marking that was renewed every 4–7 days, depending on weather conditions. All subjects were well-habituated to human presence. Due to a low culling rate (usually one individual per week), the subjects included in this study were available for the whole data collection period.

A1.2.2. Observational Data, Operational Definitions, and Video Analysis

Videos were collected on the study animals on a daily basis (except in case of heavy rain), from 06:30 a.m. to 05:00 p.m. HD video records were collected by 2–3 operators each day—from different angles—to ensure broader or different visual ranges—using Panasonic HC-V380/V180 and Sony HDR-PJ240E video recorders. In total, 224 videos were collected, including one or more individuals, corresponding to 42.67 h of video observation (Mean $\pm$ SD/subject: 4.84 ± 1.85 h). From the videos we extracted 168 aggressive events and the occurrence of behaviors was recorded in 3 min blocks in different conditions before and after each event. From the videos we also recorded the occurrence of the same behaviors under the control condition (MC). All the conditions are described below.

The video-analysis started after a training phase with both supervisors (IN, GC), when interobserver reliability scores measured *via* Cohen's k reached 0.81. The inter-observer reliability between video coders was calculated using the R function “cohen.cappa” and libraries “irr” and “psych” (R version 3.5.3). The video-analyses were carried out *via* freeware VLC 3.0.6 and extension Jump-to-Time.

We considered as stressful events the aggressive encounters resulting in physical contact between opponents [aggressive behaviors are described in **Table 1** (Bolhuis et al., 2005)].

Table1. Affinitive, aggressive, and (possible) anxiety behaviors considered in this study [integrated or modified from Bolhuis et al. (2005), Petersen et al. (1989), Stolba and Wood Gush (1989), Giersing and Andersson, (1998); Jensen, (2002); Sekiguchi and Koketsu, (2004)].

Behavioural pattern	Description
AFFINITIVE BEHAVIOURS (Figure 1)	
Rest in contact	Two subjects sit or lay in contact with one another
Social touching	A subject touches another with a paw or other body parts, except nose/head
Nose-body contact	A subject makes contact with another with its nose (via pushing or touching). For the purpose of this study, this also includes when the subjects touch each other's nose
Head-over	A subject puts its head above the back of another individual, followed by rest in contact or body contact
Nosing-body	Two subjects sniff each other on head, genitals, nose, and/or other body parts
AGGRESSIVE BEHAVIOURS	
Aggressive lifting	A subject attempts to displace another by lifting or levering it with snout or head
Aggressive biting	A subject opens its mouth and close its teeth tight on another subject's small piece of flesh, including tail
Aggressive mounting	A subject force another individual to move away by rising upon the rear of another subject

Aggressive kicking	A subjects projects of one or both hind limbs towards another subject, striking it
Aggressive pushing	A subject presses its head, neck, shoulder or body against another subject, causing the other individual to move.
Aggressive chasing	A subject pursues another subject, which flees
Aggressive head-knocking	A subject lurches or jerks its head hitting another subject
Fighting	Two subjects mutually push one another in a head to head orientation. The pattern often involve body-to-body rotation and can include aggressive mounting, lifting, biting, attempt biting, kicking, chasing, pushing, head knocking, high pitched vocalisation, with no interruption lasting more than 10s.
DISPLACEMENT ACTIVITIES	
Body scratching/rubbing	A subject uses its legs or a substrate (e.g. tree trunk) to rub part of its body
Vacuum-chewing	A subject chews with empty mouth
Head/Body shaking	A subject vigorously shakes its head and/or body (not following wallowing or similar behaviour)
Yawning	A subject performs deep, long inhalation with open mouth

For the reasons explained in the introduction, the behaviors possibly associated with transient anxiety (hereafter, target behaviors) in this study were *vacuum-chewing*, *yawning*, *head/body shaking*, and *body scratching/rubbing* (see **Table 1** for a detailed description). These behaviors are “events” (*sensu* Altmann, 1974); i.e., instantaneous patterns with no appreciable duration.

The social affiliation behaviors considered in this study are described in **Table 1** and examples are shown in **Figure 1**. Inter-individual brief contact occurring by chance during foraging (over food items naturally available in the area) was not considered as affiliation and was excluded from the analyses. None of the affiliation behaviors considered prevented the animals from performing displacement activities.



Figure 1. Examples of affiliative behaviors considered in this study. **(A)** nose-body contact; **(B)** social touching (body contact); **(C)** head-over; **(D)** rest-in-contact; **(E)** nosing body. Photo **(B)** was extracted from a video; Photos **(A,C–E)** by Ivan Norscia.

The involvement of the subjects in aggressive encounters was coded as follows: (i) aggressor: the individual actively starting the agonistic interaction by attacking another individual (*via* at least one aggressive behavior described in [Table 1](#)); (ii) aggression recipient: any individual receiving the aggressive behavior or directly receiving physical contact from an aggressor during the aggressive event (e.g., in case of multiple aggressive events or aggression redirected by the recipient of a former aggression toward another subject); (iii) bystander: any individual present on the video during an aggressive event that received no physical contact with the opponents.

Via the all occurrences sampling method (Altmann, 1974), all the behaviors detailed in Table 1 were collected from all individuals present in a video record when they were clearly visible, not sleeping and outside the periods when food was provided. This behavioral data was collected in 3-min blocks for each individual appearing in the video, falling under four conditions sharing the same or similar social context: before the stressful event (BA), 3 min after the stressful event in absence (AA) or in presence

(AP) of affiliation, and in a matched-control condition (to assess behavioral baseline levels; MC). Following De Waal and Yoshihara (1983), each matched control was paired with an observation around a conflict event and carried out during the next possible day in a similar context as the other conditions (similar weather and same time slot) but in the absence of perturbing events (aggression, sudden loud noises, food distribution, etc.) in the minutes right before the start of data collection in the conditions described above (we used 15-min as a buffer time window). In case of multiple aggressive events over a 3-min time slot, the time count started after the end of the last event. The double control (BA and MC)—compared with the other conditions—ensured that the observed behavioral variation could be directly linked to a stressful event. The three-min time window was selected for two reasons: (1) to ensure that the neuro-hormonal stress response linked to anxiety behavior was activated (Becker and Rohleder, 2019) and, (2) that the onset of the behavior directly followed the last aggressive event with no other perturbing event occurring in-between.

A1.2.3. Statistical Elaboration

Temporal Analyses. Owing to the non-normal distribution of the tested variables (Kolmogorov-Smirnov: $N_{\text{subjects}} = 37\text{--}44$, $0.001 < p \leq 0.041$) we applied different non-parametric tests at the individual animal level to check for changes in frequency of the target behaviors (Siegel and Castellan, 1988). In particular, we applied the Friedman's test for $k \geq 2$ dependent samples *via* Monte Carlo randomization to carry out sequential analyses on the target behaviors (yawning, vacuum chewing, head/body shaking, and body scratching/rubbing) expressed by the study subjects ($N_{\text{subjects}} = 44$) across conditions (BA, AA, AP, MC). We applied the Dunn *post-hoc* test for pairwise comparisons, with the significance level of probability (fixed at 0.05) adjusted downward using the Bonferroni correction.

Next, we applied the same tests (Friedman's test and pairwise Bonferroni-Dunn *post-hoc* test) to check for variation in the levels of target behaviors across the 3 min following an aggressive event.

Subsequently, we applied the Wilcoxon's test for paired samples *via* Monte Carlo randomization to carry out a minute-by-minute analysis across the 3 min following a stressful event. *Via* this test we checked for differences in target behavior occurrence before (AA) and after affiliation (AP) in the first, second, and third minute after the aggressive event. The occurrence was calculated as the number of behaviors normalized over the events for each condition. The test was run on the individuals showing both conditions (presence/absence of affiliation; $N_{\text{subjects}} = 37$) in each of the 3 min. The Monte Carlo randomization (10,000 permutations) was applied to account for possible pseudo replication (same individual involved in different behaviors during different 1-min units) or pseudo-independency (more behaviors observed after the same aggressive event which may not be fully independent). Tests were run using SPSS 20.0.

Factors Possibly Linked to Anxiety Behavior Occurrence. We ran a Generalized Linear Mixed Model (GLMM) on the aggressive events (involving $N = 74$ individuals) to verify if different individual factors and the role played by the subject in the aggression could have an effect on the occurrence of anxiety related behavior. The occurrence of the target behavior (yawning, vacuum chewing, head/body shaking, and body scratching/rubbing) was entered as dependent, binary variable (coded as presence = 1, absence = 0). The fixed factors included in the full model were the following: (i) sex (factorial variable: M = males; F = females), (ii) age (numeric variables); (iii) breed (factorial variable: PB = Parma Black, L = Landrace, PIB = Piedmont Black; (iv) involvement of the subject in the aggression (A = aggressor, R = recipient, B = bystander). Because pigs were of mixed-breeds, the breed was assigned based on the mother's. The distinction of different categories depending of subject involvement was applied only for this test. The subject identity was included as random factor.

The models were fitted in R (R Core Team, 2019; version 3.5.3) by using the function lmer of the R-package lme4 (Bates et al., 2015). As a first step it was verified if the full model significantly differed from the null model, including only the random factors (Forstmeier and Schielzeth, 2011). The likelihood ratio test (Dobson, 2002) was used to test this significance (ANOVA with argument "Chisq"). Subsequently, by using the R-function "drop1," the p-values for the individual predictors based on likelihood ratio tests between the full and the null model were calculated (Barr et al., 2013). As the response variable was binary, a binomial error distribution was used (link function: logit).

A multiple contrast package (multcomp) was used to perform all pairwise comparisons for each involvement category of significant fixed factors with the Tukey test (Bretz et al., 2010). The Bonferroni-adjusted p-values were reported, along with estimate (Est), standard error (S.E.), and z-values.

A1.3 Results

Yawning. The yawning frequencies were significantly different across the conditions considered: before the aggression (BA; Mean $\pm$ SD: 0.012 ± 0.032), after the aggression in the absence of affiliative contact (AA; 0.080 ± 0.134), after affiliative contact (AP; 0.018 ± 0.055), and in the Matched Control (MC; 0.010 ± 0.037) (Friedman test: $N = 44$, $\chi^2 = 33.224$, $df = 3$, $p < 0.001$). The pairwise comparisons (*via* Bonferroni-Dunn *post-hoc* test) revealed a significant difference between BA and AA ($Q = -0.773$, $p = 0.030$), and MC and AA ($Q = 0.852$, $p = 0.012$). A trend of significance was observed between AP and AA ($Q = 0.693$, $p = 0.071$). The difference was not significant between the other conditions (**Figure 2A**). Yawning significantly increased after exposure to an aggressive event and tended to decrease after affiliation, although not significantly.

Head/Body Shaking. The frequencies of head-body shaking were significantly different across the conditions BA (Mean $\pm$ SD: 0.010 ± 0.034), AA (0.117 ± 0.130), AP (0.057 ± 0.235), and MC (0.028 ± 0.078) (Friedman test *via* Monte Carlo randomization: $N = 44$, $\chi^2 = 34.030$, $df = 3$, $p < 0.001$). The pairwise comparisons (*via* Bonferroni-Dunn *post-hoc* test) revealed a significant difference between BA and AA ($Q = -0.955$, $p = 0.003$), AP and AA ($Q = 0.864$, $p = 0.010$), and MC and AA ($Q = 0.818$, $p = 0.018$) but not between the other conditions (**Figure 2B**). Thus, the levels of head/body shaking significantly increased after an aggressive event and the frequency of such behavior was reduced to baseline levels after affiliation (**Figure 2B**).

Scratching/Rubbing. The frequencies of scratching/rubbing were significantly different across the conditions BA (Mean $\pm$ SD: 0.012 ± 0.032), AA (0.111 ± 0.122), AP (0.059 ± 0.139), and MC (0.029 ± 0.065) (Friedman test *via* Monte Carlo randomization: $N = 44$, $\chi^2 = 26.749$, $df = 3$, $p < 0.001$). The pairwise comparisons (*via* Bonferroni-Dunn *post-hoc* test) revealed a significant difference between BA and AA ($Q = -0.966$, $p = 0.003$), AP and AA ($Q = 0.727$, $p = 0.049$), and MC and AA ($Q = 0.807$, $p = 0.020$). No difference was found between the other conditions (**Figure 2C**). The levels of body scratching/rubbing significantly increased after an aggressive event and were reduced to baseline levels after affiliative contact (**Figure 2C**).

Vacuum Chewing. The frequencies of vacuum chewing were significantly different across the conditions BA (Mean $\pm$ SD: 0.010 ± 0.052), AA (0.288 ± 0.686), AP (0.025 ± 0.102), and MC (0.016 ± 0.061) (Friedman test *via* Monte Carlo randomization: $N = 44$, $\chi^2 = 35.574$, $df = 3$, $p < 0.001$). The pairwise comparisons (*via* Bonferroni-Dunn *post-hoc* test) revealed a significant difference between BA and AA ($Q = -0.773$, $p = 0.030$), AP and AA ($Q = 0.773$, $p = 0.030$), and AA and MC ($Q = 0.727$, $p = 0.049$) but not between the other conditions (**Figure 2D**). Hence, the levels of vacuum chewing significantly increased after an aggressive event and were reduced to baseline levels after affiliation (**Figure 2D**).

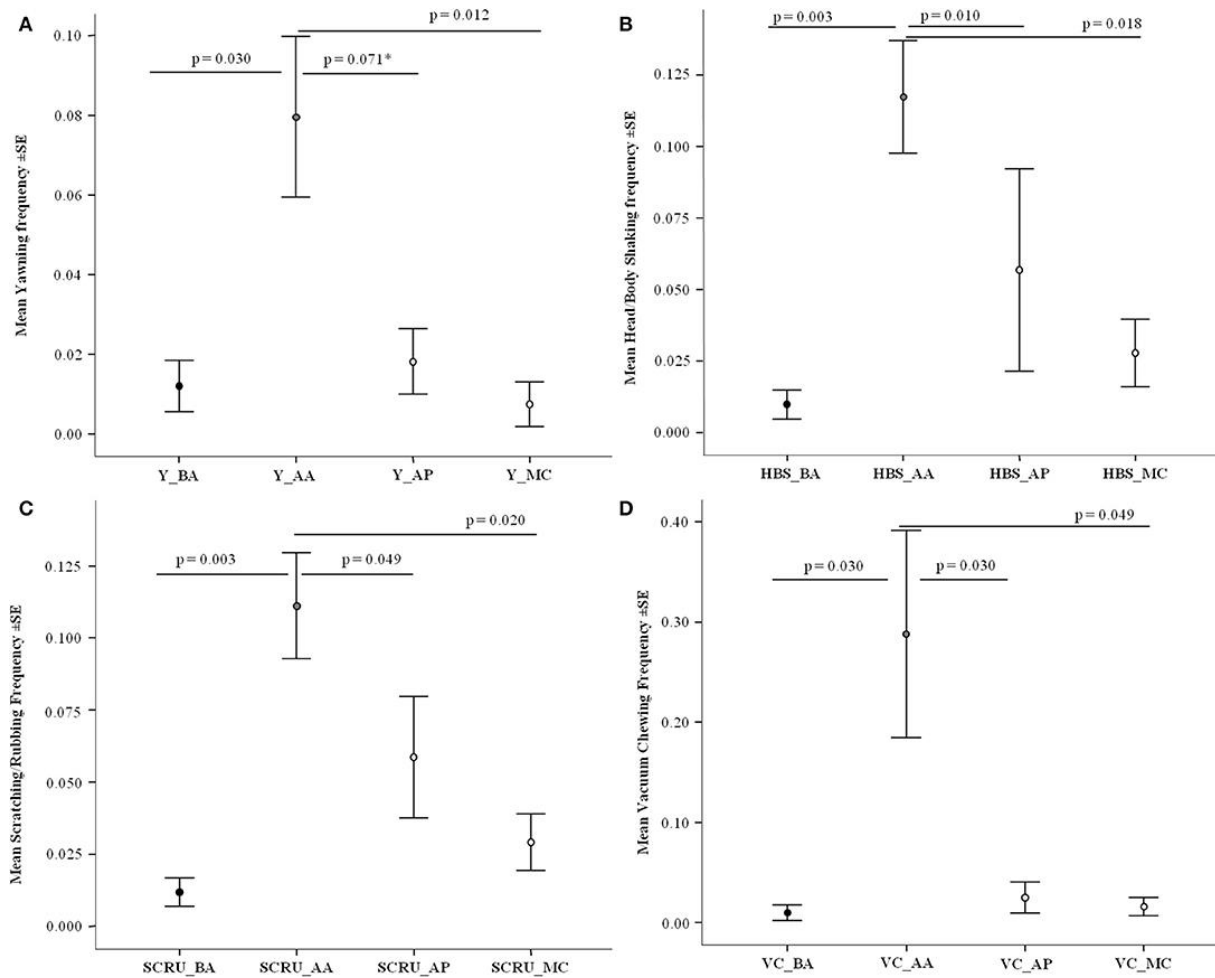


Figure 2. Anxiety-related behavior frequency (Y axis) as a function of four conditions (X axis): before aggression (BA), after aggression in absence (AA) and in presence of affiliation (AP), and matched control (no event; MC). In all cases (**A–D**) the difference across conditions was significant (Friedman's test: $26.749 \leq \chi^2 \leq 35.574$, $p < 0.001$). Behavior considered and pairwise comparison results *via* Bonferroni-Dunn test: (**A**) Yawning (Y)—Significant differences: BA/AA ($Q = -0.773$, $p = 0.030$), MC/AA ($Q = 0.852$, $p = 0.012$); trend: AP/AA ($Q = 0.693$, $p = 0.071$); not significant: BA/AP ($Q = -0.080$, $p = 1.000$), MC/BA ($Q = 0.080$, $p = 1.000$), MC/AP ($Q = 0.159$, $p = 1.000$); (**B**) Head/body shaking (HBS)—Significant differences: BA/AA ($Q = -0.955$, $p = 0.003$), AP/AA ($Q = 0.864$, $p = 0.010$), MC/AA ($Q = 0.818$, $p = 0.018$); not significant: BA/AP ($Q = -0.091$, $p = 1.000$), BA/MC ($Q = -0.136$, $p = 1.000$), AP/MC ($Q = -0.045$, $p = 1.000$); (**C**) Body scratching/rubbing (SCRU)—Significant differences: BA/AA ($Q = -0.966$, $p = 0.003$), AP/AA ($Q = 0.727$, $p = 0.049$), and MC/AA ($Q = 0.807$, $p = 0.020$); not significant: BA/MC ($Q = -0.159$, $p = 1.000$), BA/AP ($Q = -0.239$, $p = 1.000$), MC/AP ($Q = 0.080$, $p = 1.000$); (**D**) Vacuum chewing (VC)—Significant differences: BA/AA ($Q = -0.773$, $p = 0.030$), AP/AA ($Q = 0.773$, $p = 0.030$), AA/MC ($Q = 0.727$, $p = 0.049$); not significant: BA/AP ($Q = 0.000$, $p = 1.000$), BA/MC ($Q = -0.045$, $p = 1.000$), AP/MC ($Q = -0.045$, $p = 1.000$). Vertical bars: Standard Error (SE) around the mean (circles). The asterisk marks the trend of significance ($0.5 \leq p \leq 0.1$).

Minute by Minute Analysis. The level of target behaviors significantly decreased over the 3 min following an aggressive event (Friedman test *via* Monte Carlo randomization: $N = 37$, $\chi^2 = 28.777$, $df = 2$, $p < 0.001$; Mean $\pm$ SD, min 1: 0.527 ± 0.385 ; min 2: 0.290 ± 0.424 ; min 3: 0.137 ± 0.316), and particularly between minute 1 to the other minutes (min 1 vs. min 2: $Q = -0.662$, $p = 0.013$; min 1 vs. min 3: $Q =$

-1.122, $p < 0.001$) but not between minutes 2 and 3 ($Q = -0.459$, $p = 0.144$). The minute by minute analysis carried out across the 3 min following the stressful event revealed a significant difference in the levels of anxiety related behavior before (AE) and after affiliation (AA) in the first, second and third minute after the aggressive event (Wilcoxon's test *via* Monte Carlo randomization, $N = 37$; 1-min: $T = 92.00$, $p = 0.001$; 2-min: $T = 53.00$, $p = 0.029$; 3-min: $T = 0.000$, $p = 0.004$). In particular, in each given minute, the levels of anxiety related behavior were lower after affiliation than when affiliation did not occur (**Figure 3**).

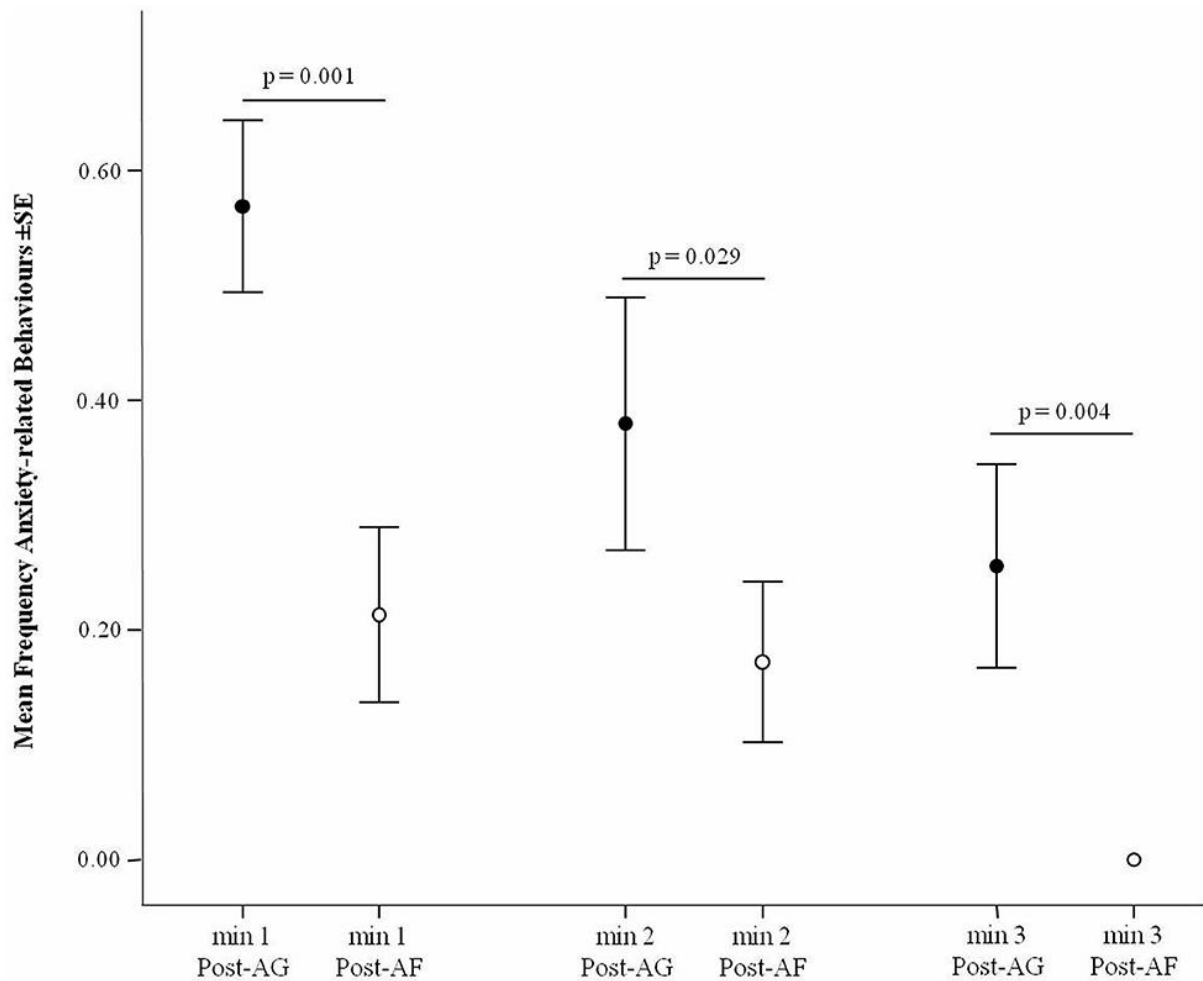


Figure 3. Frequency of anxiety-related behavior (Y axis) after aggression (Post-AG) and after affiliation (Post-AF) in each of the 3 min following the aggression (X axis). Vertical bars: Standard Error (SE) around the mean (circles).

Factors Influencing Anxiety Behavior. In the GLMM analysis, we found a significant difference between the full model versus a null model (likelihood ratio test: $N = 172$, $\chi^2 = 58.384$, $df = 6$, $p < 0.001$). Hence, we moved on with a drop1 procedure. The GLMM indicated a significant effect of the involvement of the subject in the agonistic event on the occurrence of anxiety behavior (**Table 2**). The other variables had no significant main effect. Specifically, the pairwise comparisons

revealed that anxiety behavior was more likely to occur in the aggression recipients than in the aggressors (Tukey test: Est = -1.917; S.E. = 0.719, $z = -2.667$, $p = 0.020$) and in the bystanders than in the aggression recipients (Tukey test: Est = 2.397; S.E. = 0.630, $z = 3.806$, $p < 0.001$) and aggressors (Tukey test: Est = 4.313; S.E. = 0.946, $z = 4.559$, $p < 0.001$) (**Figure 4**).

Table2. Results of the GLMM, including the following fixed factors: sex (factorial variable: M, males; F, females), age (scale variable), breed (factorial variable: PB, Parma Black; L, Large White; P, Piedmont Black), involvement of the subject in the aggression (R, recipient; A, aggressor; B, bystander).

Fixed factors	Estimate	SE	z-value	P
Intercept ^a	-1.364	1.286	<i>a</i>	<i>a</i>
Sex (M) ^b	-0.111	0.545	-0.204	0.838
Age	0.065	0.072	0.907	0.365
Breed (PB) ^b	0.141	0.807	0.175	0.861
Breed (L) ^b	0.931	0.765	1.172	0.241
Involvement (A) ^b	-1.916	0.719	-2.667	0.008
Involvement (B) ^b	2.396	0.630	3.806	<0.001

^aNot shown as not having a meaningful interpretation

^bEstimate $\pm$ SE refers to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor

The coded identity of the subject (nominal) was included as random factor. Full versus null model: $\chi^2 = 58.384$, $df = 6$, $p < 0.001$

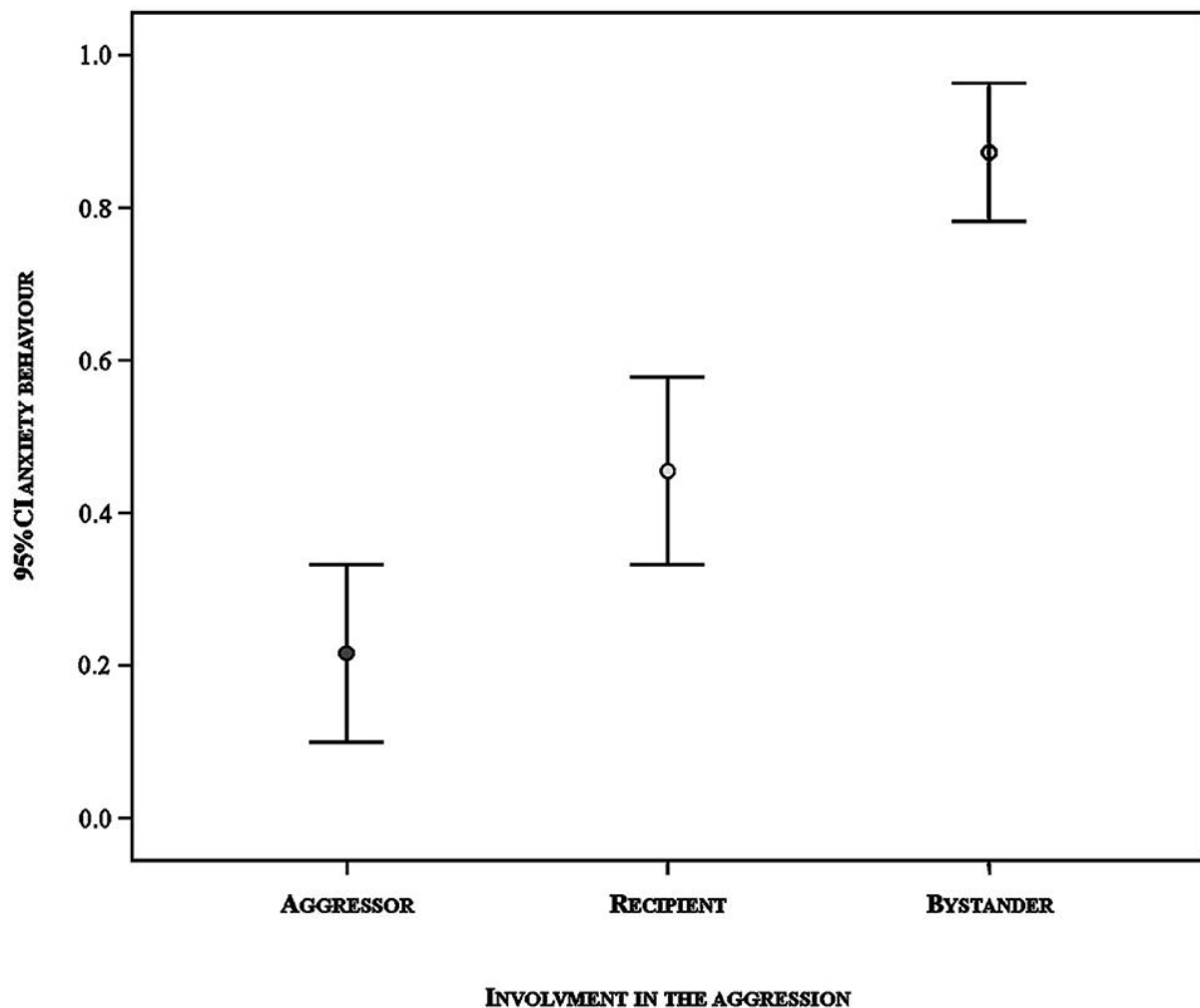


Figure 4. Likelihood of displacement activities in relation to the involvement of the subject in the aggression: aggressor, aggression's recipient and bystander. Vertical bars: 95% confidence interval around the mean (circles).

A1.4 Discussion

The results of this study show for the first time that certain behaviors, part of the typical repertoire of *Sus scrofa*, can express anxiety, be buffered by social contacts, and possibly anticipate an imminent threat. Yawning, scratching/rubbing, head/body shaking, and vacuum chewing were found to express anxiety in our study subjects because such behaviors significantly increased after a stressful intra-group aggression (Prediction 1 confirmed; **Figure 2**). The frequencies of these behaviors decreased (or in case of yawning tended to decrease) to baseline levels—and did so faster in each of the 3 min following aggression—after that affiliative contacts occurred between group mates, which indicates the presence of a social buffering mechanism (Prediction 2 confirmed; **Figures 2, 3**). Finally, the occurrence of anxiety behavior varied depending on the different level of involvement of the subjects in aggression, thus suggesting that the behavioral expression of anxiety probably is not an all-or-nothing phenomenon but can depend on the level of threat that the subjects face (Prediction 3 confirmed; **Table 2; Figure 4**). Intriguingly, our results show that after aggression, bystanders showed higher frequencies of anxiety related behavior than the individuals directly involved in the aggression (**Figure 4**), which suggests the ability of domestic pigs to anticipate, at

least in the short term, the potential threat (aggression risk) that a tense situation bears. Here below we discuss these results in detail.

A1.4.1. Anxiety Related Behavior

The fact that scratching/rubbing, head/body shaking, yawning, and vacuum chewing increased after a stressful event is in line with most of the ethological and physiological findings on other species. Self-directed behavior, including scratching, has been found to increase in tense situations in humans (Troisi, 2002; Fried, 1994) and several other mammalian species [e.g., chimpanzees, *Pan troglodytes* (Baker and Aureli, 1997); long-tailed macaque, *Macaca fascicularis* (Schino et al., 1996); Olive baboons, *Papio anubis* (Castles et al., 1999); brown lemur, *Eulemur rufusxcollaris* (Palagi and Norscia, 2010); ring-tailed lemur, *Lemur catta* (Sclafani et al., 2012); dogs (Cannas et al., 2014)]. Shaking—which can include different body parts depending on the species—or trembling has been reported as a sign of anxiety increase in humans (Bögels and Reith, 1999) and dogs (Parthasarathy and Crowell-Davis, 2006), although it was not found to increase after stressful events in horses (Scopa et al., 2018). Yawning has been associated with anxiety in humans (Guggisberg et al., 2010) and other mammalian species [South American sea lions, *Otaria flavescens* (Palagi et al., 2019); Verreaux' sifaka, *Propithecus verreauxi* and ring-tailed lemurs (Zannella et al., 2015); rats (Kubota et al., 2014)]. Vacuum chewing can increase or be associated with stressful events in horses (Kaiser et al., 2006; Lesimple et al., 2012; Scopa et al., 2018), although in horses this variation is not necessarily associated with stress-related variation of heart rate parameters (Scopa et al., 2018). This result is possibly due to the fact that vacuum chewing in horses may be associated not just with stress-related anxiety but also with positive emotions (Briefer et al., 2015; Scopa et al., 2018). Finally, vacuum chewing and yawning have been reported in pigs as stereotypic, abnormal behavior associated with chronic stress (Arellano et al., 1992; Lawrence and Terlouw, 1993; Straw et al., 1997; Scipioni et al., 2009; Zhang et al., 2017). Our results show that the behaviors considered in this study as part of the typical repertoire of the species can indicate a transient anxiety increase in ecologically sound contexts and in the absence of sources of chronic stress, such as confinement and overcrowding.

Physiological studies, combined with ethological observation and pharmacological manipulation, have provided insights on the mechanisms underlying anxiety behaviors. In particular, such studies have shown the link between the stress response mediated by glucocorticoids and scratching in mammals [dogs (Cannas et al., 2014); primates (Schino et al., 1996; Troisi, 2002; Sanders and Akiyama, 2018; Rinaldi, 2019)]. An analogous link has been demonstrated in pigs for stereotypic vacuum chewing (Zhang et al., 2017), indirectly suggested in dogs for body shaking (Beerda et al., 2000), and described in rats (Kubota et al., 2014) and hypothesized in humans for yawning (Thompson, 2011, 2014). Our results suggest that a similar association might be present in domestic pigs for the anxiety-related behaviors considered in this study. Further studies linking hormonal and behavioral variations are necessary to confirm this hypothesis.

A2.4.2. Social Buffering of Anxiety Behavior

As a second point, our results show that the social buffering of anxiety is present in domestic pigs living in naturalistic conditions because social affiliation with group mates decreased the level of anxiety-related behaviors, restoring baseline levels. For yawning, a decrease trend was observed after affiliation. This result might indicate that social buffering works also for yawning, but more data are necessary to confirm this. Alternatively, this result may be due to the fact that yawning is associated not just with an increase in anxiety levels, but more generally to physiological and behavioral changes, such as those occurring over the sleep-wake cycle or during the transition between stress arousal and relaxation (Guggisberg et al., 2010; Zannella et al., 2015). Hence, yawning can be expressed regardless of the direction of transition (e.g., from relaxation to anxiety or from anxiety to relaxation). Affinitive contacts can activate hormonal responses leading to calm restoration after anxiety arousal (Keverne et al., 1989; Brown et al., 2009; Smith and Wang, 2014). Thus, in our case, the relaxing effect of social affiliation may have buffered a decrease in yawning.

With regards to the other behaviors considered in this study (head/body shaking, vacuum chewing, and scratching/rubbing), our results show that their frequency decreased to baseline levels following affinitive contacts between group mates.

Our results show that the levels of anxiety related behavior decreased over the 3 min following the aggressive event with a peak occurring in the first minute. The minute-by-minute analysis showed that the decrease of anxiety behavior was not only due a physiological decline occurring from the time aggression elapsed. In each of the three consecutive minutes following the aggressive event, the frequency of anxiety behavior was significantly lower in the presence of, rather than in the absence of affiliation (**Figure 3**). Hence, affiliative behavior boosted the reduction of anxiety behavior in the domestic pigs under study from the first minute. This result is consistent with previous research on pigs showing that they are sensitive to the social presence or vocal support of conspecifics (Fraser, 1974; Geverink et al., 1998; Ruis et al., 2001) and that the simple proximity with group mates can reduce stress (Reimert et al., 2014). More in general, our results confirm that positive social interactions can be crucial for the health and well-being of domestic animals (Rault, 2019).

The reduction of anxiety behavior after affiliation has been observed in different social mammals, and particularly primates. For example, self-directed behavior can decrease after grooming in the ring-tailed lemur (Sclafani et al., 2012) and after playful social contacts in the common marmosets [*Callithrix jacchus* (Norscia and Palagi, 2011)], a New World monkey species. In humans, social affiliation can lead to anxiety reduction and better performance (Albas, 1990). Conciliatory contacts between former opponents were found to work in reducing anxiety behavior in brown lemurs (Palagi and Norscia, 2010), several Old World monkey species [olive baboons, *Papio anubis* (Castles and Whiten, 1998); macaques, *Macaca* spp. (Aureli and van Schaik, 1991; Das et al., 1998)], wallabies (Fraser et al., 2010), and domestic goats (Schino, 1998). Affinitive contact with a group member different from the aggressor was found to reduce post-conflict anxiety behavior in the victim of aggression in chimpanzees (Koski et al., 2007; but see Kutsukake and Castles, 2001) and bonobos (Clay and de Waal, 2013; Palagi and Norscia, 2013).

The effectiveness of social interactions in reducing anxiety behavior in domestic pigs probably reflects the importance of social relationships in this species. As a matter of fact, immature individuals of this species start establishing inter-individual

relationships via play first with littermates and later also with other conspecifics (Newberry and Wood-Gush, 1986; Weller et al., 2019; Cordoni et al., 2020). Socialization in early life also provides individuals with greater confidence and agonistic skills, which help to reduce the negative effects of aggression (Camerlink et al., 2018). In the wild counterpart, long-term relationships persist between adult females (Podgórski et al., 2014). Affiliation can be promoted by an increase of stress-related anxiety (possibly mediated by oxytocin) and produce a calming effect (Price, 2003; Mogilski et al., 2019). From an evolutionary perspective, it may have been favored to avoid the disruption of social groups. Group disruption clearly has a negative impact on the chances of survival for individuals living in social settings, considering that inter-individual interactions are crucial to obtain cooperation from others, provide protection from environmental threats, and enhance better recovery from aversive experiences (Kikusui et al., 2006). The importance of social interactions, not limited to the domesticated form, may not be strictly connected to domestication. The evolutionary history of *Sus scrofa* started long before (around 20 million years ago) and has lasted much longer than its domestication process, which began around 10,000 years ago (Larson et al., 2007; Frantz et al., 2016). It is likely that certain features of the biology of *Sus scrofa* that are found in both the domestic and the wild form (such as social traits related to survival increase) have emerged and have been favored by natural selection before artificial selection (operated by humans during domestication) came into play. Our results confirm the importance of inter-individual affiliation and social buffering in domestic pigs, as a mean to accelerate a decrease in anxiety and to quickly restore group homeostasis and individual welfare.

A1.4.3 Anxiety Behavior and Level of Threat

Our results indicate no significant effect of age, breed, and sex on the occurrence of anxiety related behavior after aggression. The fact that all individuals were adult, mixed breed, and that males were castrated may have dampened possible differences in the behavioral expression of anxiety. However, our findings indicate that the behavioral expression of anxiety in domestic pigs can vary depending on the level of threat, real, or potential, that the individuals face. In the study subjects, anxiety behavior was more expressed in the recipients of aggression, physically involved in the aggression in a defensive way, than in aggressors, which played an active role in attacking other individuals (**Figure 4**). This is in line with previous literature [but for example see (Aureli, 1997; Cordoni and Norscia, 2014)] which tends to indicate, on a general scale, that submissive subjects experience more stress-related anxiety than aggressive individuals [rats (Veenema and Neumann, 2007); ring-tailed lemurs (Sclafani et al., 2012); olive baboons (Sapolsky and Ray, 1989; Virgin and Sapolsky, 1997); humans (van Goozen, 2005; Mogilski et al., 2019)]. On a smaller scale, when considering single aggressive events, leading a fight might help release anxiety in domestic pigs in the same way as in primates (Norscia and Palagi, 2016). This anxiety-relieving effect might possibly be due to the divergent effect of testosterone and cortisol, which seems to be involved in aggressive approach and fearful withdrawal, respectively (Montoya et al., 2012).

Our findings also indicate that bystanders also expressed anxiety behavior after a conflict. The fact that anxiety behavior can increase in uninvolved individuals has been previously reported in primates, for example in the Old World monkeys *Papio hamadryas* (Judge and Mullen, 2005; Judge and Bachmann, 2013) and *Mandrillus sphinx* (Schino and Sciarretta, 2015) in which displacement activities increased in the

individuals that were only witnessing a fight and not taking part in it. In canids and primates, bystanders can be implicated in post-conflict dynamics in various ways (e.g., by interacting aggressively or in an affiliative way with aggressor and/or aggressor's recipient) to restore social cohesiveness, reduce anxiety in others, or for self-protection [e.g., (Call et al., 2002; Cools et al., 2008; Koski and Sterck, 2009; Palagi and Cordoni, 2009; Fraser et al., 2010; Romero et al., 2011; Schino and Marini, 2012; Clay and de Waal, 2013; Palagi and Norscia, 2013; Palagi et al., 2018)]. This implication of bystanders in conflicts in different species suggests that the spread of social tension following aggression may be common in many social mammals (Schino and Sciarretta, 2015). In our study group pigs that witnessed an aggression did not just show an increase in anxiety behaviors; they also showed the highest level of increase compared to those individuals directly involved in aggression. This result draws attention to at least two aspects that may deserve future detailed investigation.

The first is that anxiety and fear are two intertwined but different emotional states, which in both humans and rodents can involve overlapping but also different areas of the brain (Shin and Liberzon, 2010; Lezak et al., 2017). Fear is a response to short, present, aversive cues leading, for example, to fleeing behavior (Stankowich and Blumstein, 2005; Lezak et al., 2017). Anxiety, on the other hand, is a psychological, physiological, and behavioral state induced in animals by an imminent or potential threat (Steimer, 2002), leading to displacement activities (Troisi, 2002). Hence, recipients of aggression, when under attack, may flee (for a present aversive cue) whereas bystanders may stay and experience anxiety arousal linked to the imminent, potential threat posed by the intra-group aggression involving others. In this study, we could not investigate fear responses, as the pigs experienced very low disturbance and very rarely faced actual fearful situations. Redirected aggression from former opponents (especially recipients) to bystanders has been found to occur after conflicts in mammals, for example in primates (Butovskaya et al., 2015; Watts et al., 2000) and wolves (Palagi and Cordoni, 2009; Baan et al., 2014; Lazzaroni et al., 2017). Thus, the threat of aggression can be related to the possibility that conflict involving other subjects triggers an aggressive chain reaction hitting other group members. In the pigs of our study group, the likely different emotional states experienced by the actual recipients of aggression (victims) and potential recipients (bystanders) may account for the differences in the frequencies of anxiety behavior observed in these two categories. Further studies on pig emotional states and more generally on animal emotions are necessary to fully discern between the behavioral markers of fear and anxiety. The second aspect concerns the possible threat anticipation in domestic pigs. The additional anxiety behavior shown by bystanders compared to involved subjects points toward the possibility that pigs are able to emotionally respond to the imminent threat posed by an aggression. This observation is in line with previous findings showing that *Sus scrofa* possess short-term anticipatory skills, detected in other domains. Specifically, pigs were found to reduce aggression and increase play in anticipation of enrichment (Dudink et al., 2006) and increase their activity levels in anticipation of food (Imfeld-Mueller and Hillmann, 2012). Moreover, pigs seem to be able to share the physiological state of others [yawn contagion and emotional contagion (Reimert et al., 2013; Norscia et al., 2021)]. Moreover, pigs seem to be able

to not only mimic the emotional behavior of conspecifics and share the emotional states of others, but also synchronize their emotions with pigs that are responding emotionally in anticipation of future events (Reimert et al., 2013, 2014, 2015).

In conclusion, this study provides the first eco-ethological insight on how domestic pigs living in a natural habitat can behaviorally express non-pathological anxiety—also in anticipation of potential imminent threats—and how such anxiety can be socially buffered. These aspects are worth considering for extensive farming management under a welfare perspective. It is important to take into account not only anxiety behavior but also whether it is ensured that the animals are provided with enough space to avoid situations perceived as risky, allowing them to properly engage in positive social interactions, useful for buffering negative emotional states. Finally, owing to the ecological context in which the animals lived and the absence of abnormal or stereotypic behaviors, this study can also provide the ground work for a better understanding of the social traits that are hard wired in the species' biology and that may be the result of evolutionary convergences between domestic pigs and other highly cognitive mammals, such as human and non-human primates.

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Ethical Statement: This research was purely observational and no animal manipulation was required during the study. Hence, no ethical approval was necessary according to current regulations.

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A1.5 References

Albas, C. M. (1990). Anxiety and affiliation (or isolation?): A note on Teichman's study. *Social Behavior and Personality: An International Journal*, 18(1), 115–119. <https://doi.org/10.2224/sbp.1990.18.1.115>

- Altmann, J. (1974). Observational study of behavior sampling methods. *Behaviour*, 49(3), 227–265. <https://doi.org/10.1163/156853974X00534>
- Arellano, P. E., Pijoan, C., Jacobson, L. D., & Algers, B. (1992). Stereotyped behaviour, social interactions and suckling pattern of pigs housed in groups or in single crates. *Applied Animal Behaviour Science*, 35(2), 157–166. [https://doi.org/10.1016/0168-1591\(92\)90006-W](https://doi.org/10.1016/0168-1591(92)90006-W)
- Aureli, F., Cords, M., & Van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: A predictive framework. *Animal Behaviour*, 64(3), 325–343. <https://doi.org/10.1006/anbe.2002.3071>
- Aureli, F., & Van Schaik, C. P. (1991). Post-conflict behaviour in long-tailed macaques (*Macaca fascicularis*): II. Coping with the uncertainty. *Ethology*, 89(2), 101–114. <https://doi.org/10.1111/j.1439-0310.1991.tb00297.x>
- Aureli, F., & Yates, K. (2010). Distress prevention by grooming others in crested black macaques. *Biology Letters*, 6(1), 27–29. <https://doi.org/10.1098/rsbl.2009.0513>
- Aureli, F. (1997). Post-conflict anxiety in nonhuman primates: The mediating role of emotion in conflict resolution. *Aggressive Behavior*, 23(5), 315–328. [https://doi.org/10.1002/\(SICI\)1098-2337\(1997\)23:5<315::AID-AB2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1098-2337(1997)23:5<315::AID-AB2>3.0.CO;2-H)
- Baan, C., Bergmüller, R., Smith, D. W., & Molnar, B. (2014). Conflict management in free-ranging wolves (*Canis lupus*). *Animal Behaviour*, 90, 327–334. <https://doi.org/10.1016/j.anbehav.2014.01.033>
- Baker, K. C., & Aureli, F. (1997). Behavioural indicators of anxiety: An empirical test in chimpanzees. *Behaviour*, 134(8), 1031–1050. <https://doi.org/10.1163/156853997X00386>
- Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, 68(3), 255–278. <https://doi.org/10.1016/j.jml.2012.11.001>
- Barros, M., & Tomaz, C. (2002). Non-human primate models for investigating fear and anxiety. *Neuroscience and Biobehavioral Reviews*, 26(2), 187–201. [https://doi.org/10.1016/S0149-7634\(01\)00064-1](https://doi.org/10.1016/S0149-7634(01)00064-1)
- Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2015). *Package lme4*. Available online at: <http://lme4.r-forge.r-project.org/>
- Becker, L., & Rohleder, N. (2019). Time course of the physiological stress response to an acute stressor and its associations with the primacy and recency effect of the serial position curve. *PLoS ONE*, 14(3), e0213883. <https://doi.org/10.1371/journal.pone.0213883>
- Beerda, B., Schilder, M. B., Van Hooff, J. A., De Vries, H. W., & Mol, J. A. (2000). Behavioural and hormonal indicators of enduring environmental stress in dogs. *Animal Welfare*, 9(1), 49–62.
- Beery, A. K., & Kaufer, D. (2015). Stress, social behavior, and resilience: Insights from rodents. *Neurobiology of Stress*, 1, 116–127. <https://doi.org/10.1016/j.ynstr.2014.10.004>

Blanchard, D. C., Hynd, A. L., Minke, K. A., Minemoto, T., & Blanchard, R. J. (2001). Human defensive behaviors to threat scenarios show parallels to fear- and anxiety-related defense patterns of non-human mammals. *Neuroscience and Biobehavioral Reviews*, 25(7), 761–770. [https://doi.org/10.1016/S0149-7634\(01\)00056-2](https://doi.org/10.1016/S0149-7634(01)00056-2)

Bögels, S. M., & Reith, W. (1999). Validity of two questionnaires to assess social fears: The Dutch Social Phobia and Anxiety Inventory and the Blushing, Trembling and Sweating Questionnaire. *Journal of Psychopathology and Behavioral Assessment*, 21(1), 51–66. <https://doi.org/10.1023/A:1022812227606>

Bolhuis, J. E., Schouten, W. G., Schrama, J. W., & Wiegant, V. M. (2005). Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. *Applied Animal Behaviour Science*, 93(2), 213–228. <https://doi.org/10.1016/j.applanim.2005.01.006>

Bourin, M., Petit-Demoulière, B., Nic Dhonnchadha, B., & Hascöet, M. (2007). Animal models of anxiety in mice. *Fundamental and Clinical Pharmacology*, 21(6), 567–574. <https://doi.org/10.1111/j.1472-8206.2007.00526.x>

Bretz, F., Hothorn, T., & Westfall, P. (2010). *Multiple Comparisons Using R*. Boca Raton, FL: Chapman and Hall/CRC Press.

Briefer, E. F., Maigrot, A. L., Mandel, R., Freymond, S. B., Bachmann, I., & Hillmann, E. (2015). Segregation of information about emotional arousal and valence in horse whinnies. *Scientific Reports*, 5, 9989. <https://doi.org/10.1038/srep09989>

Brown, S. L., Fredrickson, B. L., Wirth, M. M., Poulin, M. J., Meier, E. A., Heaphy, E. D., et al. (2009). Social closeness increases salivary progesterone in humans. *Hormones and Behavior*, 56(1), 108–111. <https://doi.org/10.1016/j.yhbeh.2009.03.022>

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science*, 351(6271), 375–378. <https://doi.org/10.1126/science.aac4785>

Butovskaya, M. L., Meishvili, N. V., & Chalyan, V. G. (2015). Redirection of aggression and consolation in hamadryas baboons. *Neuroscience and Behavioral Physiology*, 45(4), 417–422. <https://doi.org/10.1007/s11055-015-0091-y>

Call, J., Aureli, F., & de Waal, F. B. M. (2002). Postconflict third party affiliation in stump-tailed macaques. *Animal Behaviour*, 63(2), 209–216. <https://doi.org/10.1006/anbe.2001.1908>

Camerlink, I., Farish, M., D'Eath, R. B., Arnott, G., & Turner, S. P. (2018). Long term benefits on social behaviour after early life socialization of piglets. *Animals*, 8(11), 192. <https://doi.org/10.3390/ani8110192>

Camerlink, I., Turner, S. P., Ursinus, W. W., Reimert, I., & Bolhuis, J. E. (2014). Aggression and affiliation during social conflict in pigs. *PLoS ONE*, 9(11), e113502. <https://doi.org/10.1371/journal.pone.0113502>

Cannas, S., Frank, D., Minero, M., Aspesi, A., Benedetti, R., & Palestini, C. (2014). Video analysis of dogs suffering from anxiety when left home alone and treated with clomipramine. *Journal of Veterinary Behavior*, 9(2), 50–57. <https://doi.org/10.1016/j.jveb.2013.12.002>

Castles, D. L., Whiten, A., & Aureli, F. (1999). Social anxiety, relationships and self-directed behaviour among wild female olive baboons. *Animal Behaviour*, 58(6), 1207–1215. <https://doi.org/10.1006/anbe.1999.1250>

Castles, D. L., & Whiten, A. (1998). Post-conflict behaviour of wild olive baboons. II. Stress and self-directed behaviour. *Ethology*, 104(2), 148–160. <https://doi.org/10.1111/j.1439-0310.1998.tb00058.x>

Clay, Z., & de Waal, F. B. (2013). Bonobos respond to distress in others: consolation across the age spectrum. *PLoS ONE*, 8(12), e55206. <https://doi.org/10.1371/journal.pone.0055206>

Cools, A. K., Van Hout, A. J. M., & Nelissen, M. H. (2008). Canine reconciliation and third-party-initiated postconflict affiliation: Do peacemaking social mechanisms in dogs rival those of higher primates? *Ethology*, 114(1), 53–63. <https://doi.org/10.1111/j.1439-0310.2007.01443.x>

Cordoni, G., Gioia, M., & Norscia, I. (2020). The dark side of play: Play fighting as a substitute for real fighting in domestic pigs (*Sus scrofa*). *Animal Behaviour Processes*. <https://doi.org/10.1016/j.beproc.2022.104778>

Cordoni, G., & Norscia, I. (2014). Peace-making in marsupials: The first study in the red-necked wallaby (*Macropus rufogriseus*). *PLoS ONE*, 9(12), e86859. <https://doi.org/10.1371/journal.pone.0086859>

Cords, M., & Thurnheer, S. (1993). Reconciling with valuable partners by long-tailed macaques. *Ethology*, 93(4), 315–325. <https://doi.org/10.1111/j.1439-0310.1993.tb01212.x>

Craig, K. J., Brown, K. J., & Baum, A. (1995). Environmental factors in the etiology of anxiety. In F. E. Bloom & D. J. Kupfer (Eds.), *Psychopharmacology: The Fourth Generation of Progress* (pp. 1325–1339). Raven Press.

Crofoot, M. C. (2013). The cost of defeat: Capuchin groups travel further, faster and later after losing conflicts with neighbors. *American Journal of Physical Anthropology*, 152(1), 79–85. <https://doi.org/10.1002/ajpa.22330>

Das, M., Penke, Z., & van Hooff, J. A. R. A. M. (1998). Postconflict affiliation and stress-related behavior of long-tailed macaque aggressors. *International Journal of Primatology*, 19(1), 53–57. <https://doi.org/10.1023/A:1020354826422>

Davis, M. (1997). Neurobiology of fear responses: The role of the amygdala. *Journal of Neuropsychiatry and Clinical Neurosciences*, 9(4), 382–402. <https://doi.org/10.1176/jnp.9.3.382>

Davitz, J., & Mason, D. J. (1955). Socially facilitated reduction of fear response in rats. *Journal of Comparative and Physiological Psychology*, 47(6), 941–947. <https://doi.org/10.1037/h0046411>

de Waal, F. B., & Preston, S. D. (2017). Mammalian empathy: Behavioural manifestations and neural basis. *Nature Reviews Neuroscience*, 18(8), 498–509. <https://doi.org/10.1038/nrn.2017.72>

de Waal, F. B. (2000). Primates—a natural heritage of conflict resolution. *Science*, 289(5479), 586–590. <https://doi.org/10.1126/science.289.5479.586>

de Waal, F. B. M., & Yoshihara, D. (1983). Reconciliation and redirected affection in rhesus monkeys. *Behaviour*, 85(3), 224–241. <https://doi.org/10.1163/156853983X00237>

Dobson, A. J. (2002). *An Introduction to Generalized Linear Models* (2nd ed.). Chapman and Hall/CRC Press.

Dudink, S., Simonse, H., Marks, I., de Jonge, F. H., & Spruijt, B. M. (2006). Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Applied Animal Behaviour Science*, 101(1), 86–101. <https://doi.org/10.1016/j.applanim.2005.12.008>

Ferdowsian, H. R., Durham, D. L., Kimwele, C., Kranendonk, G., Otali, E., Akugizibwe, T., et al. (2011). Signs of mood and anxiety disorders in chimpanzees. *PLoS ONE*, 6(12), e19855. <https://doi.org/10.1371/journal.pone.0019855>

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: Overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology*, 65(1), 47–55. <https://doi.org/10.1007/s00265-010-1038-5>

Frantz, L., Meijaard, E., Gongora, J., Haile, J., Groenen, M. A., & Larson, G. (2016). The evolution of Suidae. *Annual Review of Animal Biosciences*, 4, 61–85. <https://doi.org/10.1146/annurev-animal-021815-111155>

Fraser, D. (1974). The vocalizations and other behaviour of growing pigs in an open field test. *Applied Animal Ethology*, 1(1), 3–16. [https://doi.org/10.1016/0304-3762\(74\)90003-0](https://doi.org/10.1016/0304-3762(74)90003-0)

Fraser, O. N., Stahl, D., & Aureli, F. (2008). Stress reduction through consolation in chimpanzees. *Proceedings of the National Academy of Sciences of the United States of America*, 105(22), 8557–8562. <https://doi.org/10.1073/pnas.0804141105>

Fraser, O. N., Stahl, D., & Aureli, F. (2010). The function and determinants of reconciliation in *Pan troglodytes*. *International Journal of Primatology*, 31(1), 39–57. <https://doi.org/10.1007/s10764-009-9377-7>

Fried, R. G. (1994). Evaluation and treatment of psychogenic pruritus and self-excoriation. *Journal of the American Academy of Dermatology*, 30(6), 993–999. [https://doi.org/10.1016/S0190-9622\(94\)70125-3](https://doi.org/10.1016/S0190-9622(94)70125-3)

Geverink, N. A., Bühnemann, A., van de Burgwal, J. A., Lambooij, E., Blokhuis, H. J., & Wiegant, V. M. (1998). Responses of slaughter pigs to transport and lairage sounds. *Physiology & Behavior*, 63(5), 667–673. [https://doi.org/10.1016/S0031-9384\(97\)00513-1](https://doi.org/10.1016/S0031-9384(97)00513-1)

Giersing, M., & Andersson, A. (1998). How does former acquaintance affect aggressive behaviour in repeatedly mixed male and female pigs? *Applied Animal Behaviour Science*, 59(4), 297–306. [https://doi.org/10.1016/S0168-1591\(98\)00141-5](https://doi.org/10.1016/S0168-1591(98)00141-5)

Gordon, J. A., & Hen, R. (2004). Genetic approaches to the study of anxiety. *Annual Review of Neuroscience*, 27, 193–222. <https://doi.org/10.1146/annurev.neuro.27.070203.144212>

Guggisberg, A. G., Mathis, J., Schnider, A., & Hess, C. W. (2010). Why do we yawn? *Neuroscience & Biobehavioral Reviews*, 34(8), 1267–1276. <https://doi.org/10.1016/j.neubiorev.2010.03.008>

Hachisuka, J., Chiang, M. C., & Ross, S. E. (2018). Itch and neuropathic itch. *Pain*, 159(4), 603. <https://doi.org/10.1097/j.pain.0000000000001141>

Hennessy, M. B., Maken, D. S., & Graves, F. C. (2000). Consequences of the presence of the mother or unfamiliar adult female on cortisol, ACTH, testosterone, and behavioral responses of periadolescent guinea pigs during exposure to novelty. *Psychoneuroendocrinology*, 25(6), 619–632. [https://doi.org/10.1016/S0306-4530\(00\)00014-7](https://doi.org/10.1016/S0306-4530(00)00014-7)

Imfeld-Mueller, S., & Hillmann, E. (2012). Anticipation of a food ball increases short-term activity levels in growing pigs. *Applied Animal Behaviour Science*, 137(1–2), 23–29. <https://doi.org/10.1016/j.applanim.2012.01.012>

Jeffcoate, W. J., Lincoln, N. B., Selby, C., & Herbert, M. (1986). Correlation between anxiety and serum prolactin in humans. *Journal of Psychosomatic Research*, 30(3), 217–222. [https://doi.org/10.1016/0022-3999\(86\)90052-8](https://doi.org/10.1016/0022-3999(86)90052-8)

Jensen, P. (2002). Behaviour of pigs. In P. Jansen (Ed.), *The Ethology of Domestic Animals* (pp. 159–172). Wallingford: CABI Publishing.

Judge, P. G., & Bachmann, K. A. (2013). Witnessing reconciliation reduces arousal of bystanders in a baboon group (*Papio hamadryas hamadryas*). *Animal Behaviour*, 85(5), 881–889. <https://doi.org/10.1016/j.anbehav.2013.02.011>

Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, 69(6), 1345–1355. <https://doi.org/10.1016/j.anbehav.2004.08.016>

Kaiser, L., Heleski, C. R., Siegford, J., & Smith, K. A. (2006). Stress-related behaviors among horses used in a therapeutic riding program. *Journal of the American Veterinary Medical Association*, 228(1), 39–45. <https://doi.org/10.2460/javma.228.1.39>

Kaiser, S., Hennessy, M. B., & Sachser, N. (2015). Domestication affects the structure, development, and stability of biobehavioural profiles. *Frontiers in Zoology*, 12(S1), S19. <https://doi.org/10.1186/1742-9994-12-S1-S19>

Keverne, E. B., Martensz, N. D., & Tuite, B. (1989). Beta-endorphin concentrations in cerebrospinal fluid of monkeys are influenced by grooming relationships. *Psychoneuroendocrinology*, 14(2), 155–161. [https://doi.org/10.1016/0306-4530\(89\)90065-6](https://doi.org/10.1016/0306-4530(89)90065-6)

Kikusui, T., Winslow, J. T., & Mori, Y. (2006). Social buffering: Relief from stress and anxiety. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 361(1467), 2215–2228. <https://doi.org/10.1098/rstb.2006.1941>

Koski, S. E., Koops, K., & Sterck, E. H. M. (2007). Reconciliation, relationship quality, and post-conflict anxiety: Testing the integrated hypothesis in captive chimpanzees. *American Journal of Primatology*, 69(2), 158–172. <https://doi.org/10.1002/ajp.20338>

Koski, S. E., & Sterck, E. H. M. (2009). Post-conflict third-party affiliation in chimpanzees: What's in it for the third party? *American Journal of Primatology*, 71(5), 409–418. <https://doi.org/10.1002/ajp.20668>

Kubota, N., Amemiya, S., Yanagita, S., Nishijima, T., & Kita, I. (2014). Emotional stress evoked by classical fear conditioning induces yawning behavior in rats. *Neuroscience Letters*, 566, 182–187. <https://doi.org/10.1016/j.neulet.2014.02.064>

Kutsukake, N., & Castles, D. L. (2001). Reconciliation and variation in post-conflict stress in Japanese macaques (*Macaca fuscata fuscata*): Testing the integrated hypothesis. *Animal Cognition*, 4(4), 259–268. <https://doi.org/10.1007/s10071-001-0119-2>

Larson, G., Albarella, U., Dobney, K., Rowley-Conwy, P., Schibler, J., Tresset, A., et al. (2007). Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 104(31), 15276–15281. <https://doi.org/10.1073/pnas.0703411104>

Latham, N. R., & Mason, G. J. (2008). Maternal deprivation and the development of stereotypic behaviour. *Applied Animal Behaviour Science*, 110(1–2), 84–108. <https://doi.org/10.1016/j.applanim.2007.03.026>

Lawrence, A. B., & Terlouw, E. C. (1993). A review of behavioral factors involved in the development and continued performance of stereotypic behaviors in pigs. *Animal Science*, 71(11), 2815–2825. <https://doi.org/10.2527/1993.71102815x>

Lazzaroni, M., Marshall-Pescini, S., & Cafazzo, S. (2017). Post-conflict opponent affiliation reduces victim re-aggression in a family group of captive arctic wolves (*Canis lupus arctos*). *PLoS ONE*, 12(12), e0187450. <https://doi.org/10.1371/journal.pone.0187450>

Lesimple, C., Sankey, C., Richard, M. A., & Hausberger, M. (2012). Do horses expect humans to solve their problems? *Frontiers in Psychology*, 3, 306. <https://doi.org/10.3389/fpsyg.2012.00306>

Lezak, K. R., Missig, G., & Carlezon, W. A., Jr. (2017). Behavioral methods to study anxiety in rodents. *Dialogues in Clinical Neuroscience*, 19(2), 181. <https://doi.org/10.31887/DCNS.2017.19.2/wcarlezon>

Maestriperieri, D., Schino, G., Aureli, F., & Troisi, A. (1992). A modest proposal: Displacement activities as an indicator of emotions in primates. *Animal Behaviour*, 44(6), 967–979. [https://doi.org/10.1016/S0003-3472\(05\)80592-5](https://doi.org/10.1016/S0003-3472(05)80592-5)

Malik, J. S., Singh, P., Beniwal, M., & Kumar, T. (2019). Prevalence of depression, anxiety, and stress among jail inmates. *International Journal of Community Medicine and Public Health*, 6(4), 1306–1309. <https://doi.org/10.18203/2394-6040.ijcmph20190631>

Marino, L., & Colvin, C. M. (2015). Thinking pigs: A comparative review of cognition, emotion, and personality in *Sus domesticus*. *International Journal of Comparative Psychology*, 28, 1–22. <https://doi.org/10.46867/ijcp.2015.28.00.04>

Martínez-Miró, S., Tecles, F., Ramón, M., Escribano, D., Hernández, F., Madrid, J., et al. (2016). Causes, consequences and biomarkers of stress in swine: An update. *BMC Veterinary Research*, 12, 171. <https://doi.org/10.1186/s12917-016-0791-8>

McCoy, C. R., Jackson, N. L., Day, J., & Clinton, S. M. (2017). Genetic predisposition to high anxiety- and depression-like behavior coincides with diminished DNA methylation in the adult rat amygdala. *Behavioral Brain Research*, 320, 165–178. <https://doi.org/10.1016/j.bbr.2016.12.008>

McFarland, R., & Majolo, B. (2011). Reconciliation and the costs of aggression in wild Barbary macaques (*Macaca sylvanus*): A test of the integrated hypothesis. *Ethology*, 117, 928–937. <https://doi.org/10.1111/j.1439-0310.2011.01948.x>

Mogilski, J. K., Wysocki, A., Reeve, S. D., Mitchell, V. E., Lunge, J., & Welling, L. L. (2019). Stress hormones, physiology, and behavior. In W. L. Welling & T. K. Shackelford (Eds.), *The Oxford Handbook of Evolutionary Psychology and Behavioral Endocrinology* (pp. 351–371). Oxford University Press.

Montoya, E. R., Terburg, D., Bos, P. A., & Van Honk, J. (2012). Testosterone, cortisol, and serotonin as key regulators of social aggression: A review and theoretical perspective. *Motivation and Emotion*, 36, 65–73. <https://doi.org/10.1007/s11031-011-9264-3>

Muráni, E., Ponsuksili, S., D'Eath, R. B., Turner, S. P., Kurt, E., Evans, G., et al. (2010). Association of HPA axis-related genetic variation with stress reactivity and aggressive behaviour in pigs. *BMC Genetics*, 11, 74. <https://doi.org/10.1186/1471-2156-11-74>

Neumann, I. D., Veenema, A. H., & Beiderbeck, D. I. (2010). Aggression and anxiety: Social context and neurobiological links. *Frontiers in Behavioral Neuroscience*, 4, 12. <https://doi.org/10.3389/fnbeh.2010.00012>

Newberry, R. C., & Wood-Gush, D. G. M. (1986). Social relationships of piglets in a semi-natural environment. *Animal Behaviour*, 34, 1311–1318. [https://doi.org/10.1016/S0003-3472\(86\)80202-0](https://doi.org/10.1016/S0003-3472(86)80202-0)

Norscia, I., Coco, E., Robino, C., Chierito, E., & Cordoni, G. (2021). Yawn contagion in domestic pigs (*Sus scrofa*). *Scientific Reports*, 11, 1851. <https://doi.org/10.1038/s41598-020-80545-1>

Norscia, I., & Palagi, E. (2011). When play is a family business: Adult play, hierarchy, and possible stress reduction in common marmosets. *Primates*, 52, 101–104. <https://doi.org/10.1007/s10329-010-0228-0>

Norscia, I., & Palagi, E. (2016). *The Missing Lemur Link: An Ancestral Step in the Evolution of Human Behaviour* (Vol. 74). Cambridge University Press.

Palagi, E., & Cordoni, G. (2009). Postconflict third-party affiliation in *Canis lupus*: Do wolves share similarities with the great apes? *Animal Behaviour*, 78, 979–986. <https://doi.org/10.1016/j.anbehav.2009.07.017>

Palagi, E., Guillén-Salazar, F., & Llamazares-Martín, C. (2019). Spontaneous yawning and its potential functions in South American sea lions (*Otaria flavescens*). *Scientific Reports*, 9, 1–8. <https://doi.org/10.1038/s41598-019-53613-4>

Palagi, E., Leone, A., Demuru, E., & Ferrari, P. F. (2018). High-ranking geladas protect and comfort others after conflicts. *Scientific Reports*, 8, 1–12. <https://doi.org/10.1038/s41598-018-33548-y>

Palagi, E., & Norscia, I. (2013). Bonobos protect and console friends and kin. *PLoS ONE*, 8, e79290. <https://doi.org/10.1371/journal.pone.0079290>

Palagi, E., & Norscia, I. (2010). Scratching around stress: Hierarchy and reconciliation make the difference in wild brown lemurs (*Eulemur fulvus*). *Stress*, 14, 93–97. <https://doi.org/10.3109/10253890.2010.505272>

Parthasarathy, V., & Crowell-Davis, S. L. (2006). Relationship between attachment to owners and separation anxiety in pet dogs (*Canis lupus familiaris*). *Journal of Veterinary Behavior*, 1, 109–120. <https://doi.org/10.1016/j.jveb.2006.09.005>

Petersen, H. V., Vestergaard, K., & Jensen, P. (1989). Integration of piglets into social groups of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 23, 223–236. [https://doi.org/10.1016/0168-1591\(89\)90113-5](https://doi.org/10.1016/0168-1591(89)90113-5)

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., & Jedrzejewska, B. (2014). Long-lasting, kin-directed female interactions in a spatially structured wild boar social network. *PLoS ONE*, 9, e99875. <https://doi.org/10.1371/journal.pone.0099875>

Price, J. S. (2003). Evolutionary aspects of anxiety disorders. *Dialogues in Clinical Neuroscience*, 5, 223. <https://doi.org/10.31887/DCNS.2003.5.3/jprice>

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: <https://www.R-project.org/>

Rault, J. L. (2019). Be kind to others: Prosocial behaviours and their implications for animal welfare. *Applied Animal Behaviour Science*, 210, 113–123. <https://doi.org/10.1016/j.applanim.2018.10.015>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: Emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, 18, 517–532. <https://doi.org/10.1007/s10071-014-0820-6>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109, 42–50. <https://doi.org/10.1016/j.physbeh.2012.11.002>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology & Behavior*, 129, 221–229. <https://doi.org/10.1016/j.physbeh.2014.02.059>

Rinaldi, G. (2019). The itch-scratch cycle: A review of the mechanisms. *Dermatology Practical & Conceptual*, 9, 90. <https://doi.org/10.5826/dpc.0902a03>

Romero, T., Castellanos, M. A., & de Waal, F. B. (2011). Post-conflict affiliation by chimpanzees with aggressors: Other-oriented versus selfish political strategy. *PLoS ONE*, 6, e22173. <https://doi.org/10.1371/journal.pone.0022173>

Ruis, M. A., de Groot, J., te Brake, J. H., Ekkel, E. D., van de Burgwal, J. A., Erkens, J. H., et al. (2001). Behavioural and physiological consequences of acute social defeat in growing gilts: Effects of the social environment. *Applied Animal Behaviour Science*, 70, 201–225. [https://doi.org/10.1016/S0168-1591\(00\)00150-7](https://doi.org/10.1016/S0168-1591(00)00150-7)

Salonen, M., Sulkama, S., Mikkola, S., Puurunen, J., Hakanen, E., Tiira, K., et al. (2020). Prevalence, comorbidity, and breed differences in canine anxiety in 13,700 Finnish pet dogs. *Scientific Reports*, 10, 1–11. <https://doi.org/10.1038/s41598-020-59837-z>

Sanders, K. M., & Akiyama, T. (2018). The vicious cycle of itch and anxiety. *Neuroscience & Biobehavioral Reviews*, 87, 17–26. <https://doi.org/10.1016/j.neubiorev.2018.01.009>

Sapolsky, R. M., & Ray, J. C. (1989). Styles of dominance and their endocrine correlates among wild olive baboons (*Papio anubis*). *American Journal of Primatology*, 18, 1–13. <https://doi.org/10.1002/ajp.1350180102>

Schino, G., & Marini, C. (2012). Self-protective function of post-conflict bystander affiliation in mandrills. *PLoS ONE*, 7, e38936. <https://doi.org/10.1371/journal.pone.0038936>

Schino, G., Perretta, G., Taglioni, A. M., Monaco, V., & Troisi, A. (1996). Primate displacement activities as an ethopharmacological model of anxiety. *Anxiety*, 2, 186–191. [https://doi.org/10.1002/\(SICI\)1522-7154\(1996\)2:4<186::AID-ANXI5>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1522-7154(1996)2:4<186::AID-ANXI5>3.0.CO;2-M)

Schino, G., & Sciarretta, M. (2015). Effects of aggression on interactions between uninvolved bystanders in mandrills. *Animal Behaviour*, 100, 16–21. <https://doi.org/10.1016/j.anbehav.2014.11.013>

Schino, G. (1998). Reconciliation in domestic goats. *Behaviour*, 135, 343–356. <https://doi.org/10.1163/156853998793066302>

Scipioni, R., Martelli, G., & Volpelli, A. (2009). Assessment of welfare in pigs. *Italian Journal of Animal Science*, 8, 117–137. <https://doi.org/10.4081/ijas.2009.s1.117>

Sclafani, V., Norscia, I., Antonacci, D., & Palagi, E. (2012). Scratching around mating: Factors affecting anxiety in wild *Lemur catta*. *Primates*, 53, 247–254. <https://doi.org/10.1007/s10329-012-0294-6>

Scopa, C., Palagi, E., Sighieri, C., & Baragli, P. (2018). Physiological outcomes of calming behaviors support the resilience hypothesis in horses. *Scientific Reports*, 8, 1–9. <https://doi.org/10.1038/s41598-018-35561-7>

Sekiguchi, T., & Koketsu, Y. (2004). Behavior and reproductive performance by stalled breeding females on a commercial swine farm. *Journal of Animal Science*, 82, 1482–1487. <https://doi.org/10.2527/2004.8251482x>

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, 35, 169–191. <https://doi.org/10.1038/npp.2009.83>

Siegel, S., & Castellan, N. J. (1988). *Nonparametric statistics for the behavioral sciences* (2nd ed.). McGraw-Hill.

Smith, A. S., & Wang, Z. (2014). Hypothalamic oxytocin mediates social buffering of the stress response. *Biological Psychiatry*, 76, 281–288. <https://doi.org/10.1016/j.biopsych.2013.09.017>

Stankowich, T., & Blumstein, D. T. (2005). Fear in animals: A meta-analysis and review of risk assessment. *Proceedings of the Royal Society B*, 272, 2627–2634. <https://doi.org/10.1098/rspb.2005.3251>

Steimer, T. (2002). The biology of fear- and anxiety-related behaviors. *Dialogues in Clinical Neuroscience*, 4, 231. <https://doi.org/10.31887/DCNS.2002.4.3/tsteimer>

Stolba, A., & Wood-Gush, D. G. M. (1989). The behaviour of pigs in a semi-natural environment. *Scientific Journal of Animal*, 48, 419–425. <https://doi.org/10.1017/S0003356100040411>

Straw, B. E., Zimmerman, J. J., Dallaire, S., & Taylor, D. J. (1997). *Diseases of swine* (9th ed.). Blackwell Publishing.

Thompson, S. B. (2011). Born to yawn? Cortisol linked to yawning: A new hypothesis. *Medical Hypotheses*, 77, 861–862. <https://doi.org/10.1016/j.mehy.2011.07.056>

Thompson, S. B. (2014). Yawning, fatigue, and cortisol: Expanding the Thompson Cortisol Hypothesis. *Medical Hypotheses*, 83, 494–496. <https://doi.org/10.1016/j.mehy.2014.08.009>

Thorsteinsson, E. B., James, J. E., & Gregg, M. E. (1998). Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology*, 17, 436. <https://doi.org/10.1037/0278-6133.17.5.436>

Tiira, K., & Lohi, H. (2015). Early life experiences and exercise associate with canine anxieties. *PLoS ONE*, 10, e0141907. <https://doi.org/10.1371/journal.pone.0141907>

Tinbergen, N. (1952). Derived activities; their causation, biological significance, origin, and emancipation during evolution. *Quarterly Review of Biology*, 27, 1–32. <https://doi.org/10.1086/398642>

Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress*, 5, 47–54. <https://doi.org/10.1080/102538902900012378>

van Goozen, S. H. M. (2005). Hormones and the developmental origins of aggression. In R. E. Tremblay, W. W. Hartup, & J. Archer (Eds.), *Developmental origins of aggression* (pp. 281–306). The Guilford Press.

Van Riezen, H., & Segal, M. (1988). *Comparative Evaluation of Rating Scales for Clinical Psychopharmacology*. Elsevier Science Ltd.

van Wolkenten, M. L., Davis, J. M., Gong, M. L., & de Waal, F. B. (2006). Coping with acute crowding by *Cebus apella*. *International Journal of Primatology*, 27, 1241–1256. <https://doi.org/10.1007/s10764-006-9070-z>

Veenema, A. H., & Neumann, I. D. (2007). Neurobiological mechanisms of aggression and stress coping: A comparative study in mouse and rat selection lines. *Brain, Behavior, and Evolution*, 70, 274–285. <https://doi.org/10.1159/000105491>

Virgin, C. E. Jr., & Sapolsky, R. M. (1997). Styles of male social behavior and their endocrine correlates among low-ranking baboons. *American Journal of Primatology*, 42, 25–39. [https://doi.org/10.1002/\(SICI\)1098-2345\(1997\)42:1<25::AID-AJP2>3.0.CO;2-0](https://doi.org/10.1002/(SICI)1098-2345(1997)42:1<25::AID-AJP2>3.0.CO;2-0)

Watts, D. P., Colmenares, F., & Arnold, K. (2000). Redirection, consolation, and male policing. In F. Aureli & F. B. M. de Waal (Eds.), *Natural Conflict Resolution* (pp. 281–301). University of California Press.

Weller, J. E., Camerlink, I., Turner, S. P., Farish, M., & Arnott, G. (2019). Socialisation and its effect on play behaviour and aggression in the domestic pig (*Sus scrofa*). *Scientific Reports*, 9, 1–11. <https://doi.org/10.1038/s41598-019-40980-1>

Zannella, A., Norscia, I., Stanyon, R., & Palagi, E. (2015). Testing yawning hypotheses in wild populations of two strepsirrhine species: *Propithecus verreauxi* and *Lemur catta*. *American Journal of Primatology*, 77, 1207–1215. <https://doi.org/10.1002/ajp.22459>

Zhang, M. Y., Li, X., Zhang, X. H., Liu, H. G., Li, J. H., & Bao, J. (2017). Effects of confinement duration and parity on stereotypic behavioral and physiological responses of pregnant sows. *Physiology & Behavior*, 179, 369–376. <https://doi.org/10.1016/j.physbeh.2017.07.015>

A2. Sows' Responses to Piglets in Distress: An Experimental Investigation in a Natural Setting

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Abstract

Domestic pigs (*Sus scrofa*) possess complex socio-cognitive skills, and sows show high inter-individual variability in maternal behaviour. To evaluate how females—reared under natural conditions—react to the isolation calls of their own piglets or those of other females, we conducted observations and experimental trials. In January–February 2021, we conducted all-occurrences sampling on affiliation, aggression, and lactation (daily, 7:30–16:30 h) on six lactating and four non-lactating females at the ethical farm Parva Domus (Turin, Italy). The trials (30 s each, $n = 37/\text{sow}$) consisted of briefly catching and restraining a piglet. We recorded the sow response (none/reactive/proactive movement towards the piglet; self-directed anxiety behaviours such as body shaking) before and during the trial and under control conditions. Increased levels of anxiety behaviour in sows were accompanied by an increased frequency of responses. Less aggressive sows and lactating sows showed the highest frequencies of response. Finally, the isolation calls' maximum intensity had an influence on the type of response observed, with higher proactive response frequencies following lower intensity isolation calls. Our results suggest that being under lactation could play a key role in increasing sow response levels and that specific acoustic features may influence the response.

A2.1 Introduction

In mammals, the attachment between mother and infant develops during pregnancy or immediately after delivery, and it is promoted by social cues such as communicative signals, including vocalisations (Goumon and Špinka, 2016). In different mammalian species, infants emit vocalisations when they are separated from their mothers or, more generally, when they are distressed to maintain or regain contact with their mother (Zeifman, 2001; Charrier et al., 2002; Insley et al., 2003; Newman, 2004; Torriani et al., 2006; Zeskind, 2013). Distress acoustic signals—remarkably similar across mammals (e.g., isolation calls)—may convey information on the emitter's conditions (e.g., via intensity or frequency) (Manteuffel et al., 2004; Brudzynski, 2007). Distressed and endangered infants may elicit an increase in mothers' arousal, induce mothers to increase vigilance, and establish communication with their offspring for protection (Chaiken, 1992; Ehret and Haack, 1981; Ehret, 1992; Zeskind and Collins, 1987; Nelson and Panksepp, 1998; Zeifman, 2001; Newman, 2004, 2007; Manteuffel et al., 2004; Hahn and Lavooy, 2005; Torriani et al., 2006; Brudzynski, 2007; Lingle et al., 2012; Parsons et al., 2012; Briefer, 2012; Lingle and Rendall, 2007; Lingle and Riede, 2014; Del Vecchio et al., 2009; Lonstein et al., 2015; Breedveld et al., 2019; Hiraoka et al., 2020). In sows, hormone stress levels can influence maternal behaviour (Baxter et al., 2023), although it is unclear how transient arousal (e.g., measured via anxiety behaviours) can affect maternal behaviour under natural conditions.

Pigs show behaviours that are specifically associated with physiological anxiety (scratching, head-body rubbing, head shaking, yawning, and vacuum chewing, Norscia et al., 2021). Pigs have a large vocal repertoire (Newberry and Wood-Gush, 1986; Schön et al., 1998, 1999), and acoustic communication is often used in mother–infant interactions (Fraser, 1975; Ocepek and Andersen, 2017, 2018), especially during early interactions, such as lactation and abrupt separations (Algers, 1993; Špinka et al., 2002). Piglets vocalise more when they need maternal care and show a context-dependent plasticity of acoustic signals (Weary and Fraser, 1995; Weary et al., 1997), screaming when they perceive an imminent threat (Illmann et al., 2013), and emitting different vocalisations depending on the situation. For example, distress vocalisations are emitted by piglets when they are isolated from their mother, when they need heat, or if they feel pain (Signoret et al., 1975; Weary and Fraser, 1995; Weary et al., 1996, 1997; Illmann et al., 2002, 2013). In particular, isolation calls emitted by piglets are characterised initially by quiet, low-frequency sounds and later by louder, high-frequency sounds (Signoret et al., 1975; Weary and Fraser, 1995; Weary et al., 1996, 1997; Illmann et al., 2002, 2013; Goumon et al., 2020). In playback experiments, it has been documented that sows (i) are able to recognise their offspring depending on the acoustic characteristics of the piglet calls that are typical of the litter (Špinka et al., 2002); and (ii) can show stronger responses to the acoustic signals of infants in distress, especially when the duration and intensity of piglet isolation calls are higher (Weary et al., 1996).

Different studies carried out under experimental conditions have shown that the maternal response of sows may depend not only on the acoustic properties of isolation calls (perceptual factors) but also on individual and social factors. Specifically, female pigs may develop stable social relationships, especially in the wild, and lactating sows can show allomaternal care in response to the piglets of other, especially strongly related, sows (Signoret et al., 1975; Goumon et al., 2020; Durrell et al., 2004). On the other hand, under experimental conditions, it has been observed that sows can respond more intensely to the playback of isolation calls when the acoustic signal is emitted by their own litter (Weary et al., 1996). Moreover, under experimental conditions, less aggressive sows can show stronger maternal behavioural responses (Løvendahl et al., 2005). Despite the above information, no study so far has specifically addressed the relation between arousal increase and responses of females (both mothers and non-mothers) under natural conditions in pigs and how such responses can be modulated by individual, perceptual, and social factors (considered together). To fill this gap, based on the above pieces of evidence on pigs, we formulated the general hypothesis that sows may respond to piglets' isolation calls via arousal increase, and that their response may vary depending on individual (i.e., aggressive levels, being a lactating sow/mother sow or not), perceptual (i.e., call intensity and duration), and social factors (i.e., relationship between mothers and other sows).

A2.2 Materials and Methods

A2.2.1. Study site and subjects

This study was carried out in January-February 2022 (temperature: average = $3.9^{\circ}\text{C} \pm 0.4$; min = $-0.6^{\circ}\text{C} \pm 0.4$; max: $11.6^{\circ}\text{C} \pm 0.7$; humidity: $74.3\% \pm 2.2$) at the “Ethical Farm Parva Domus”, in Cavagnolo, Turin (Italy). The sows under study (Parma Black breed) could freely move and forage in a 1.0 ha area of natural grassland/woodland habitat. Four shelters – which could accommodate multiple females - were available in the upper part of the outdoor area, spaced a few meters apart (Figure 1).



Figure 1. Sow shelters in the study area.

The animals were provisioned with food (Ciclo Unico P, SILDAMIN©) every day from 8:30 to 10:30 in a feeding zone (upper part of the area); water was available *ad libitum*. Sows could integrate their diet with roots and leaves that they could find in the environment. For individual recognition, all the individuals of the group – including piglets - were marked with non-toxic spray Raidex© for livestock or identified based on unique distinctive features (e.g. a combination of color, black or white spots, wattles, etc.). To estimate weight, we carried out a set of size measurements - including body length and chest girth – at the beginning of the data collection period. Body length and chest girth can explain around 90% of the weight variation and can therefore provide a reliable estimate of pig live weight (Reiland, 1978). The measurements were taken with the aid of a clothing tape while the subjects were feeding in a spot (with no necessity to constrain them). Body length was measured from the neck base to the tail base right above the shoulder, and chest girth was determined by measuring the circumference of the chest area behind the forelegs (Mutua et al., 2011). Weight (kg) was estimated by applying the following formula (Reiland, 1978):

$$0,39 * \text{length} + 0.64 * \text{girth}.$$

All sows (Parma Black breed; estimated average weight, lactating females: 122.03Kg $\pm$ 4.83; non-lactating females: 110.89 $\pm$ 3.43) were sexually mature (with

sexual maturity being reached at 5-6 months of age; Birteeb et al., 2015). The group was composed of 6 lactating females (1 to 1.5 years old; 3 primiparous and 3 non-primiparous) and 4 non-lactating females (7 months old). All sows were already habituated to the human presence so it was possible to easily approach and mark them from the beginning of the study. However, the first five days were used for individual identification and training on observational data collection.



Figure 2. The study group (one individual is not visible).

All the lactating sows had given birth within a period of 24 days. One sow (SR) lost her litter on the first day after birth (because the piglets had been inadvertently crushed by another female, as observed during the second day on the field) but kept lactating piglets from other litters throughout the study period. No sow showed stereotypic behaviour (e.g., repeated and/or abnormal behaviours in absence of any perturbing event). All the sows had the same father and could be either full-siblings or half-siblings. Table 1 shows the details of the sows under study including the date of delivery.

Table 1. List and characteristics of sows.

Sow	Sow category	Age (months)	Date birth	of	Litter (number of piglets)	Trials as mother	Trials as non-mother
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PB	Lactating female	18	02/01/2022	6	12	25
AAB	Lactating female	12	23/12/2021	3	12	25
AAG	Lactating female	12	15/01/2022	6	12	25
AAR	Lactating female	12	22/12/2021	8	12	25
SB	Lactating female	18	01/01/2022	8	12	25
SR*	Lactating female	18	15/01/2022	0	0	37
QQB	Non-lactating female	7	NA	NA	NA	37
QQR	Non-lactating female	7	NA	NA	NA	37
XB	Non-lactating female	7	NA	NA	NA	37
XR	Non-lactating female	7	NA	NA	NA	37

* SR lost her litter on the first day after birth.

A2.2.2. Data collection and operational definitions

Behavioural data collection was carried out on a daily basis from around 7:30 am to 4:30 pm. Around 80% of the time, sows would stay in the area surrounding the shelters so it was possible to observe them easily all together or in subgroups. Affiliation states (*sensu* Altmann, 1974; lay-in-contact and sit-in-contact) and suckling session data were gathered via audio and/or video recording by using the all-occurrences sampling method (Altmann, 1974). The observers noted which sows were not visible during observations so to be able to calculate the observation time of each sow and dyad. Individual behavioural frequencies were determined as the number of observed behavioural bouts for a given individual normalized over the observation time of that individual. The dyadic interaction frequencies for each pair of sows were determined as the number of interactions exchanged normalized over the observation time of the dyad (when the sows of the dyad could be both observed at the same time). The dyadic affiliation frequencies were used as indicators of the relationship quality between sows.

Each day we recorded all the conflicts occurring right after food provisioning by the farmer, over a time window of 15-min. For each aggression, the identity of aggressor (sow directing the first aggressive pattern to another sow) and recipient (sow receiving the first aggressive pattern) were noted. The behaviours were categorized following the ethogram used by Norscia et al. (2021) and Cordoni et al. (2022) and described in Table 2. Ethological observations were stopped when other activities were carried out (e.g., experimental trials, feeding). Observers stayed at around 10 m from the piglets and sows.

Table 2. Aggressive, affiliative and anxiety-related behaviours of domestic pig (*Sus scrofa*) considered in this study (Norscia et al., 2021; Cordoni et al., 2022).

Category	Behavioural pattern	Description
Anxiety-related behaviours	Body Scratching/rubbing	A pig uses its legs or a substrate to rub part of its body
	Vacuum-chewing	A pig chews with empty mouth
	Head/Body shaking	A pig vigorously shakes its head and/or body
	Yawning	A pig performs deep, long inhalation with open mouth
Aggressive behaviours	Aggressive lifting	A pig attempts to displace a fellow by lifting or levering it with snout or head
	Aggressive biting	A pig opens its mouth and closes its teeth tight on a fellow's small piece of flesh, including tail
	Aggressive mounting	A pig forces a fellow to move away by rising upon its rear
	Aggressive kicking	A pig projects of one or both hind limbs towards a fellow, striking it
	Aggressive pushing	A pig presses its head, neck, shoulder or body against a fellow thus causing its moving
	Aggressive chasing	A pig pursues a fellow, which flees
	Aggressive head-knocking	A pig lurches or jerks its head hitting a fellow
	Fighting	Two pigs mutually push one another in a head to head orientation. The pattern can involve body-to-body rotation and/or aggressive mounting, lifting, biting, attempt biting, kicking, chasing, pushing, head knocking, high pitched vocalisation, with no interruption lasting more than 10s
Affiliative behaviours	Rest in contact	Two pigs sit or lay in contact with one another
	Social touching	A pig touches a fellow with a paw or other body parts, except nose/head

	Nose-nose contact	A pig touches with its nose the nose of a fellow
	Nose-body contact	A pig touches/pushes with its nose a body part of a fellow (excluding nose)
	Head-over	A pig puts its head above the back of a fellow; rest in contact or body contact can after occur

A2.2.3. Experimental trials

The experimental sessions were carried out between around 12:30 and 16:30 p.m. in similar environmental conditions (no rain and in the shelter area). The experimenters wore the same external clothes across trials ([Figure 3](#)). To ensure that the sows were not in an arousal state and allow cross-individual and cross-trial comparison, the starting condition of each trial session was the following: at least one mother with litter and one non-mother had to be present within the shelter area (≤ 15 m), with sows inactive (but awake), in the absence of external perturbing events. At the time of the trials, all individuals were in the shelters or around (≤ 30 m) and not dispersed. The experimenter was always the same (L.C.) and would take position within 1 m from the shelter where at least one piglet was found. The first videographer—always the same (A.P.)—would take a position three metres away from the experimenter, directly facing him. A second videographer (I.N.) would take a position within 10 m of the other videographer when necessary and would record the sows that the first videographer could not film (off-screen sows). Distances, including the distance between the vocalising piglet and sow, were predetermined via measuring tape and barricade stripe tape (used to mark metres) to calculate the metres between shelters and between experimenters and animals. To avoid possible habituation issues, each sow was tested a maximum of three times per day.

The experimental session was composed of the following phases (**Figure 3**): (i) pre-trial phase (PRE): the experimenter and the videographers stayed still for 30 s; (ii) trial phase (TP): the experimenter caught and restrained a piglet (for max 30 s) and the sow behaviour was recorded during the period following the beginning of piglet isolation calls (characterised initially by quiet, low-frequency sounds and later by louder, high-frequency sounds); (iii) matched-control (MC): for each TP, a corresponding 30 s matched-control (MC) observation of the behaviour of the same individuals was recorded on the next possible day, in the same social and environmental context, in the absence of external perturbing events. The PRE and MC phases were used as controls to ensure that the sow behavioural change (if any) was a reaction to the isolation call emitted by the piglet and not enacted regardless. In PRE, TP, and MC, we collected data on self-directed behaviour with a previously demonstrated association with anxiety (self-scratching, body rubbing, head/body shaking, yawning, vacuum chewing; **Table 2**; Norscia et al., 2021; Cordoni et al., 2022). During TP, the maximum intensity of the isolation call (dB) was recorded using a phonometer (V-RESOURCING RZ1358; measuring range: 30–130 dBA, accuracy

within ± 1.5 dB; resolution: 0.1 dBA) positioned at a distance of 50 cm from the piglet emitting the vocalisation (emitter). To ensure data independence, the inter-trial interval was at least 3 min (the time that is required for the females to return to homeostasis as per Norscia et al., 2021), and a new trial was carried out when all the sows were again in the starting condition (see above).

The trial was considered as null if at least one of the following conditions occurred: (i) an isolation call was emitted from a subject other than the piglet caught and restrained by the experimenter; (ii) no isolation call occurred; (iii) a disturbance factor emerged (e.g., car or tractor passing close by, dog barking, etc.); (iv) if the sow moved their head or body when the experimenter was in the process of catching the piglet, before the isolation call occurred (trial invalid for the disturbed sow only). In no case the piglets showed any of the anxiety behaviours reported in **Table 2** after the 'catching' phase.

In the field, we randomly selected the sow for each trial by blindly picking the name from a pool of all sows' names (a sow name was removed from the pool when three trials were reached for that day). Trial validity was checked along the way to keep track of the valid trials. We carried out 37 valid sessions/sow out of a total of 108 sessions. In detail, for the different categories, we carried out 12 valid sessions/lactating sow, with the sow being the mother of the vocalising piglet, and 25 valid sessions/lactating sow, with the sow as a non-mother.



Figure 3. Trial sequence (top: pre-trial; middle: trial phase; bottom: MC).

A2.2.4. Operational definitions, video coding

We recognised three categories of sows: non-lactating females (gilts), lactating females that were not the mothers of the vocalising piglets (hereafter, lactating non-mothers), and mothers of the vocalising piglets (hereafter, lactating mothers). We identified three possible types of sow behavioural responses, extracted from videos: no response (the sow did not move at all), reactive responses (the sow moved her head towards the experimenter or changed position, directing her body towards the experimenter, but did not leave the spot where she stayed before the screaming), and proactive response (the sow moved towards the experimenter). In no case did the sows move away from the vocalising piglet.

Because it was not possible to predict when and for how long the piglet would vocalise in TP, for cross-phase behavioural comparisons, we normalised the response bouts over the observation seconds/phase across trials. The isolation call duration

(Mean $\pm$ SE: 10.13 s $\pm$ 8.18) was extracted from the trial videos to be tested as a possible variable affecting the sow reaction (see the ‘Statistical analyses’ section below).

The isolation call intensity was then recalculated from the maximum vocalisation intensity recorded close to the vocalisation emitter, compared to the attenuation provided by the law of the inverse distance (Berg and Stork, 2004; Pierce and Beyer, 1990) on the basis of the distance between the experimenter and the sow (vocalisation receiver), by using the formula (L_{eq} = vocalisation decibels for the receiver; L_{rif} = vocalisation decibels recorded at 50 cm to the emitter; r = linear distance between emitter and receiver; r_{rif} = distance to the emitter):

$$L_{eq} = L_{rif} - 20 * \log_{10} \frac{r}{r_{rif}}$$

The sex and age of the piglet and the distance between the vocalising piglet and the tested sow were considered control variables.

The extraction of behaviours (**Table 2**) from videos was carried out via the freeware Potplayer 1.7.21. In particular, slow motion and/or frame-by-frame analysis were applied to extract data on sow responses and anxiety behavioural events (sensu Altman, 1974). The video coding on affiliation states, conflict and anxiety events was carried out by A.P. and L.C., and the inter-observer reliability was determined on 10% of videos based on Cohen’s k for aggression, anxiety behaviours, and affiliative states (Cohen’s $0.87 \leq k \leq 0.92$). The coding of sow responses (reactive, proactive, or no response) was considered valid only in the case of 100% agreement across three observers (A.P., L.C., and E.C.) for all trials.

A2.2.5. Statistical Analyses

To verify the variation of sow behaviour (anxiety behaviours and behavioural changes at the individual level) across phases (PRE, PT, and MC), we applied a non-parametric Friedman’s test for k dependent samples (non-normal data distribution of data; Kolmogorov–Smirnov test: $N_{sows} = 10$; $0.001 \leq p \leq 0.094$). We applied the Dunn post hoc test for pairwise comparisons, with the significance level of probability adjusted downward using the Bonferroni correction.

We ran a first GLMM (GLMM₁) to verify what individual factors affected the probability of observing sow response (target variable: presence/absence of response) to the piglet isolation call ($N_{cases} = 370$). We tested the following fixed factors: (i) sow category (non-lactating female, lactating non-mother, lactating mother); (ii) sow aggressive level (non-top/top quartile). As further control fixed factors we included: (i) piglet age (days); (ii) piglet sex (male/female); (iii) distance between the vocalising piglet and sow (metres); (iv) isolation call duration category (non-top/top quartiles); (v) max decibel intensity of isolation call (non-top/top quartile); (vi) sow affiliation level

(non-top/top quartile); (vii) number of trials for each sow (numeric). The sow and piglet identities were included as random factors.

The second GLMM (GLMM₂) was restricted to the cases where a response from the sow was observed ($N_{\text{response_cases}} = 58$), to test what perceptive factors can influence the type of response. The target, dependent variable was the type of sow response (proactive if the sow moved towards the experimenter; reactive if the sow moved her head towards the experimenter or changed position directing her body towards the experimenter but did not leave the spot) to the piglet's isolation call. We tested the main effect of the following fixed factors: (i) isolation duration category (factor: 0 = non-top quartiles; 1 = top quartile); and (ii) max decibel intensity of vocalisation (factor: 0 = non-top quartiles; 1 = top quartile). The sow and piglet identities were included as random factors.

The third GLMM (GLMM₃) was carried out on the sows that were not the mother of the vocalising piglet ($N_{\text{non_mother_cases}} = 320$) to test whether the relationship between the sow and the mother of the vocalising piglet would influence the response probability. The target, dependent variable was the occurrence of the response (presence/absence) of the sow to the piglet's isolation call. We tested the main effect of the following fixed factors: (i) aggressive levels of the dyad non-mother and mother of the vocalising piglet (factor: 0 = non-top quartiles; 1 = top quartile); and (ii) affiliation levels of the same dyad (factor: 0 = non-top quartiles; 1 = top quartile). The sow and piglet identities were included as random factors.

The last GLMM (GLMM₄) was carried out on lactating females only ($N_{\text{lactating_female_cases}} = 222$) to check whether lactation variables would influence the response. The target, dependent variable was the occurrence of the response (presence/absence) of the sow to the piglet's isolation call. We tested the main effects of the following fixed factors: lactation duration (factor: 0 = non-top quartiles; 1 = top quartile); and lactation frequency (factor: 0 = non-top quartiles; 1 = top quartile). The sow and piglet identities were included as random factors.

The association between each GLMM analysis and specific assumptions derived from the general hypothesis are shown in **Table 3**.

Table 3. The association between each GLMM analysis and specific assumptions under the general hypothesis that sows may respond to piglets' isolation calls via arousal increase, with their response possibly varying depending on individual, perceptual, and social factors.

Sow Sample	Assumptions within the General Hypothesis	Analysis
All sows	1. We expected that isolation calls of piglets would cause: (1a) increased levels of anxiety-related behaviours in sows; (1b) increased frequency of behavioural responses; (1c) increased	GLMM ₁ : Individual factors possibly affecting the occurrence of sow response to piglet vocalisation ($N_{\text{cases}} = 370$). Target variable: occurrence of the response (presence/absence) of the sow to the

	frequencies of responses in mothers of the piglet that emitted the isolation calls compared to non-mothers; (1d) a higher frequency of responses by sows showing lower levels of aggression.	piglet's isolation call. Fixed factors: (i) sow category (non-lactating female, lactating non-mother, lactating mother); (ii) sow aggressive level (non-top/top quartile). Further control (fixed) factors: (i) piglet age (days); (ii) piglet sex (male/female); (iii) distance between the vocalising piglet and sow (metres); (iv) isolation call duration category (non-top/top quartiles); (v) max decibel intensity of isolation call (non-top/top quartile); (vi) sow affiliation level (non-top/top quartile); (vii) number of trials for each sow (numeric). Random factors: sow and piglet identity.
Responding sows	2. We expected that more intense and longer-lasting piglet isolation calls might elicit a higher frequency of behavioural responses from the sows.	GLMM ₂ : Perceptive factors possibly influencing the type of response ($N_{\text{response_cases}} = 58$). Target variable: type of the sow response to the piglet's isolation call (proactive/reactive). Fixed factors: (i) isolation duration category (non-top/top quartile); and (ii) max decibel intensity of vocalisation (non-top/top quartile). Random factors: sow and piglet identity.
Non-mother sows	3. We expected that the sows would respond more to vocalisations emitted by piglets that were not their offspring when they had higher affiliation levels—and lower aggressive levels—with the mother of the vocalising piglet.	GLMM ₃ : Possible influence of the relationship between the sow and the mother of the vocalising piglet on the response probability. ($N_{\text{non_mother_cases}} = 320$). Target variable: occurrence of the sow response (presence/absence). Fixed factors: (i) aggressive levels of the dyad non-mother and mother of the vocalising piglet (non-top/top quartile); (ii) affiliation levels of the same dyad (non-top/top quartile). Random factors: sow and piglet identity.
Lactating females	4. We expected to find higher levels of response in sows with higher lactation rates and duration.	GLMM ₄ : Possible effect of lactation variables on the sow response ($N_{\text{lactating_female_cases}} = 222$). Target variable: occurrence of sow response (presence/absence). Fixed factors: lactation duration (non-top/top quartile); lactation frequency (non-top/top quartile). Random factors: sow and piglet identity.

We fit the GLMMs in R (R Core Team, 2023) by using the function “glmer” of the R-package lme4 (Bates et al., 2015), and we plotted the scaled (quantile) residuals for fitted (generalised) linear mixed models by using the R-package DHARMa (Hartig,

2023). We established the significance of the full model by comparison to a null model that only included the random effects (Forstmeier and Schielzeth, 2011). We used a likelihood ratio test (Dobson and Barnett, 2018) to test this significance (ANOVA with argument 'Chisq'). We calculated the p values for the individual predictors based on likelihood ratio tests between the full and the null models by using the R-function "drop1" (Barr et al., 2013). As the target variables were binomial, a binomial error distribution was used. For significant multinomial predictors, we performed all pairwise comparisons with the Tukey test (Bretz et al., 2016) using a multiple contrast package (multcomp). We reported the Bonferroni-adjusted p values, estimate (Est), standard error (SE), and Z values.

We obtained the variance inflation factor (VIF) for the three numeric variables of GLMM₁ (distance, piglet age, and trial number) via the "vif" function in R. All VIF values were >1.00, thus indicating no collinearity. We calculated the effect size via the package "effectsize" and the function effectsize, which returns the best effect-size measure for the provided input GLMM. For all tests (except in the case of Bonferroni correction, see above), the significance probability threshold was fixed at 0.05.

A2.3 Results

There was a significant variation in the frequencies of anxiety behaviours of sows across the three phases (PRE, TP, MC; Friedman test: $N_{\text{sows}} = 10$, $\chi^2 = 11.655$, $df = 2$, $p = 0.003$). The pairwise comparisons (via Bonferroni–Dunn post hoc test) revealed a significant difference between PRE and TP ($p = 0.042$) and MC and TP ($p = 0.030$), but not between PRE and MC ($p = 1.000$). In particular, the sow showed the highest levels of anxiety behaviours in TP than in the PRE and MC phases (**Figure 4a**), thus confirming an anxiety increase following the piglet isolation call.

There was a significant variation in the behavioural response of sows across the three phases (PRE, TP, MC; Friedman test: $N_{\text{sows}} = 10$, $\chi^2 = 18.000$, $df = 2$, $p < 0.001$). The pairwise comparisons (via Bonferroni-Dunn post hoc test) revealed a significant difference between PRE and TP ($p = 0.008$) and MC and TP ($p = 0.008$), but not between PRE and MC ($p = 1.000$). In particular, the sow response was higher in the TP phase than in the PRE and MC phases (**Figure 4b**), thus indicating that such a response was likely associated with the vocalising piglets and not with other factors (e.g., the mere presence of experimenters).

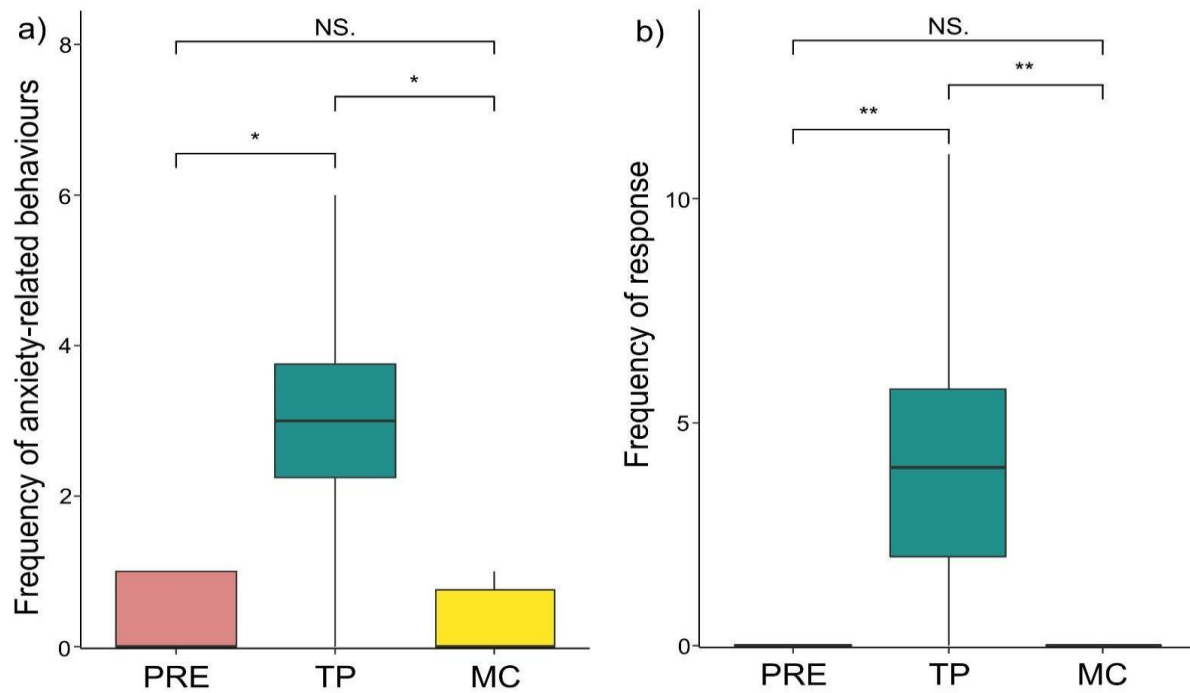


Figure 4. Box plot showing that: (a) anxiety behaviour frequencies are higher in TP, whereas there are no differences between PRE and MC (Friedman test: $N_{\text{sows}} = 10$, $\chi^2 = 11.655$, $df = 2$, $p = 0.003$; PRE vs. TP: $p = 0.042$; MC vs. TP: $p = 0.030$; PRE vs. MC: $p = 1.000$). There was an increase in anxiety-related behaviour of sows following the isolation call of vocalising piglets; (b) response frequencies are higher in TP, whereas there are no differences between PRE and MC (Friedman test: $N_{\text{sows}} = 10$, $\chi^2 = 18.000$, $df = 2$, $p < 0.001$; PRE vs. TP: $p = 0.008$; MC vs. TP: $p = 0.008$; PRE vs. MC: $p = 1.000$). There was an increase in sows response frequencies following the isolation call of vocalising piglets. Horizontal line: median value; box: interquartile range; vertical line: minimum and maximum values in the data. NS = non-significant, * = $p < 0.05$; ** = $p < 0.01$

GLMM₁ was carried out to verify the effect of individual factors on the target variable (the presence/absence of sow response). The full model (including all fixed factors and control fixed factors) and the null model (only including the random factor) significantly differ (likelihood ratio test: $\chi^2 = 31.226$; $df = 10$; $p < 0.001$). Because at least one predictor had a significant effect on the target variable, we applied the drop1 procedure. We found that the sow category and the aggression level of the sow had a significant main effect on the probability of observing a response, whereas the affiliation level of the sow and the other control fixed factors (distance, piglet age and sex, screaming duration, isolation call intensity, and trial number) did not (**Table 4**). In particular, sows that showed lower levels of aggression most likely responded to vocalising piglets (**Table 4**; **Figure 5**).

Table 4. GLMMs results: influence of individual factors (GLMM₁), relationship with the mother of the vocalising piglet (GLMM₃), and lactation variables (GLMM₄); influence of perceptual factors on the type of response (GLMM₂).

Predictors	Estimates	SEM	CI ₉₅	Effect size	χ^2	<i>p</i>
GLMM ₁	<i>n</i> _{cases} = 370; full vs. null model: $\chi^2 = 31.226$; df = 10; <i>p</i> < 0.001					
Intercept	-2.961	0.946	-3.73, 1.56	-	a	a
Sow category (lactating mother) ^b	1.912	0.630	0.68, 3.15	1.91	3.037	0.002
Sow category (non-mother) ^b	2.859	0.743	1.40, 4.31	2.86	3.849	< 0.001
Distance (from 1 to 15 m)	-0.077	0.047	-0.72, 0.07	-0.33	-1.631	0.103
Scream duration (long duration) ^b	0.120	0.422	-0.30, 0.41	0.05	0.284	0.776
Piglets sex (female) ^b	-0.645	0.379	-1.39, 0.10	-0.64	-1.703	0.089
Piglet age	0.020	0.024	-0.25, 0.62	0.19	0.844	0.399
Isolation call maximum intensity (high intensity) ^b	-0.700	0.453	-1.59, 0.19	-0.70	-1.545	0.122
Sow aggression frequency (high levels) ^b	-0.886	0.433	-1.73, 0.04	-0.89	-2.045	0.041
Sow affiliation frequency (high levels) ^b	0.537	0.724	-0.88, 1.96	0.54	0.742	0.458
Sow trial number	0.003	0.019	-0.35, 0.42	0.03	0.174	0.862
GLMM ₂	<i>n</i> _{cases} = 58; full vs. null model: $\chi^2 = 6.390$; df = 2; <i>p</i> = 0.041					
Intercept	1.533	0.432	0.69, 2.38	a	a	a
Scream duration (long duration) ^b	-0.233	0.695	-1.60, 1.13	-0.23	-0.335	0.738
Isolation call maximum intensity (high intensity) ^b	-1.590	0.652	-2.87, 0.31	-1.59	-2.439	0.015
GLMM ₃	<i>n</i> _{cases} = 320; full vs. null model: $\chi^2 = 1.323$; df = 2; <i>p</i> = 0.516					
GLMM ₄	<i>n</i> _{cases} = 222; full vs. null model: $\chi^2 = 0.325$; df = 2; <i>p</i> = 0.850					

Note: Random factor: Piglet identity and Sow identity (GLMM₁, GLMM₂, GLMM₃, GLMM₄). Bold values indicate *p* < 0.05

^aNot shown as not having a meaningful interpretation.

^bThese predictors were dummy-coded, with the reference category as follow: Sow category: “lactating non-mother”; Piglet sex: “male”; Scream duration: “short duration”; Isolation call maximum intensity: “low intensity”; Sow aggression frequency: “low levels”; Sow affiliation frequency: “low levels”.

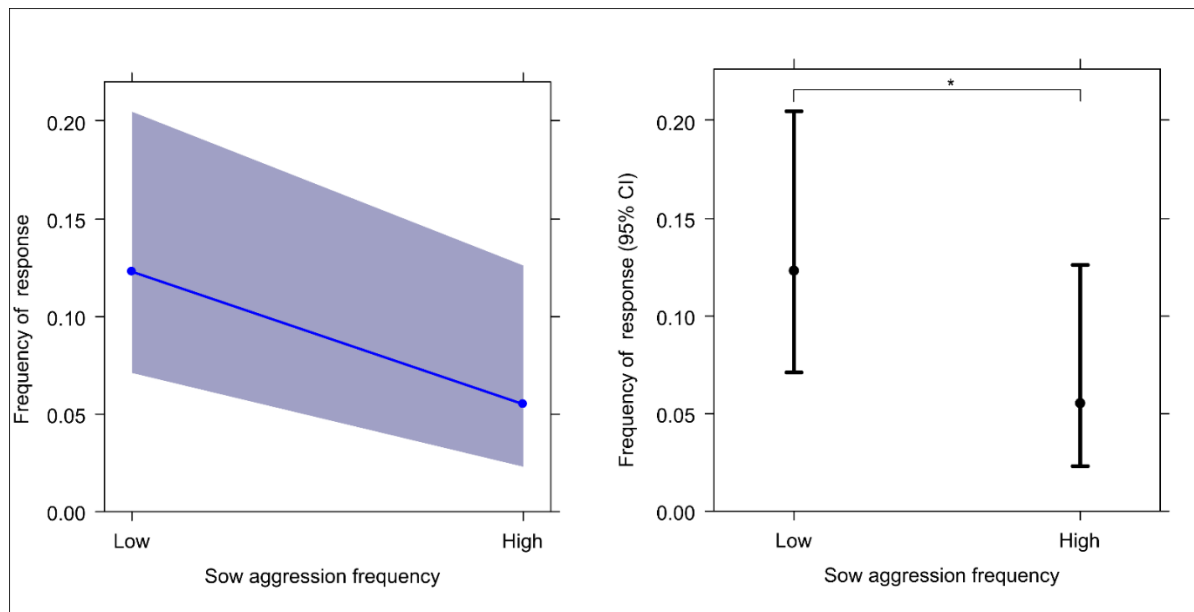


Figure 5. The effect plot (right) shows that the factor “sow aggression frequency” has a significant main effect on the target variable with the response frequency (Y axis) being higher in sows showing low aggression levels (compared to sows with high aggression levels; X axis; likelihood ratio test: $\chi^2 = 31.226$; $df = 10$; $p < 0.001$). The slope indicates the magnitude of main effect. The error bars (left) shows confidence interval (CI, black bars) around the mean (central black dot) of the response frequency (Y axis) as function of the sow aggression intensity (low/high; X axis). * = $p < 0.05$

Moreover, lactating females (either mothers or non-mothers of the vocalising piglet) responded significantly more than non-lactating females (Table 4; Figure 6; Tukey test; lactating non-mothers vs non-lactating females, Est = 1.912, SE = 0.630, $Z = 3.037$, $p = 0.006$; lactating mothers vs non-lactating females, Est = 2.859, SE = 0.743, $Z = 3.849$, $p < 0.001$). Lactating mothers did not respond significantly more frequently than lactating non-mothers (Tukey test; Est = 0.947, SE = 0.497, $Z = 1.905$, $p = 0.131$). Hence, lactation (present or not) set a major difference in the probability to observe a response by a sow to a vocalising piglet.

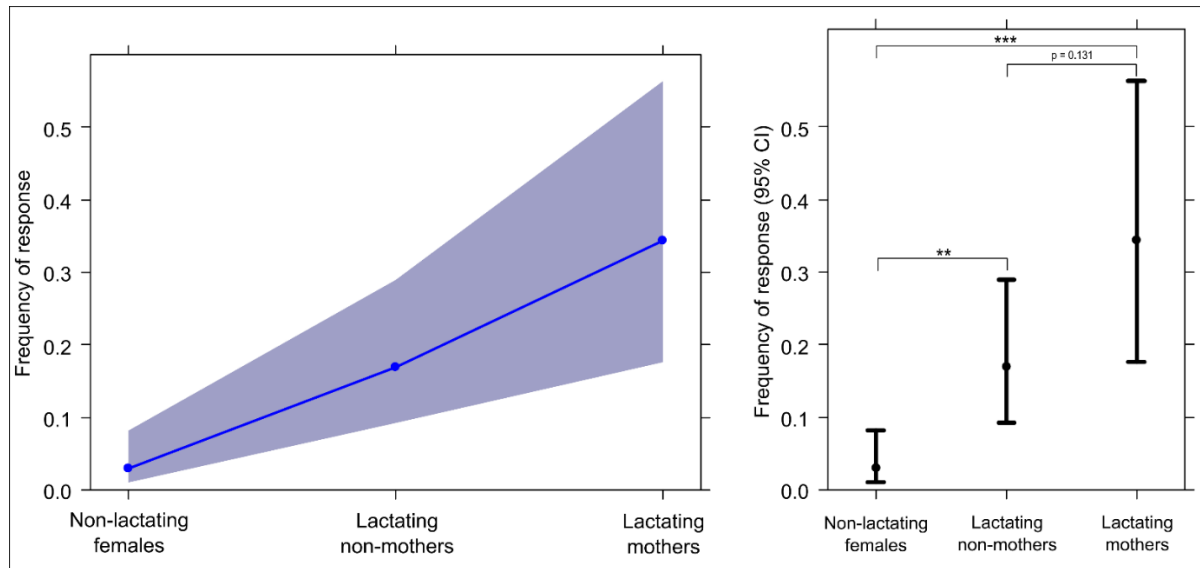


Figure 6. The effect plot (right) shows that the factor 'sow category' (non-lactating females, and lactating mothers and non mothers; X axis) has a significant main effect on the target variable with the response frequency (Y axis) being highest in lactating mothers and non-mothers likelihood ratio test: $\chi^2 = 31.226$; $df = 10$; $p < 0.001$; Tukey test; lactating non-mothers vs. non-lactating females, $p = 0.006$; lactating mothers vs. non-lactating females, $p < 0.001$; lactating mothers vs. lactating non-mothers, $p = 0.131$). The slope indicates the magnitude of main effect. The error bars (left) shows confidence interval (CI, black bars) around the mean (central black dot) of the response frequency (Y axis) as function of the sow category (non-lactating females, and lactating mothers and non mothers; X axis). ** = $p < 0.01$; *** = $p < 0.001$.

GLMM₂ was carried out on response cases to verify whether isolation call duration or maximum intensity would significantly affect the type of sow response (reactive/proactive). The full model (including all fixed factors) and the null model (only including the random factor) significantly differ (likelihood ratio test: $\chi^2 = 6.390$; $df = 2$; $p = 0.041$). Because at least one predictor had a significant effect on the target variable, we applied the drop1 procedure. The maximum isolation call intensity, but not the duration, had a significant main effect on the type of response (**Table 4**), with a proactive response being most likely observed in cases of lower maximum decibels of the vocalisation (**Figure 7**). Hence, sows passively reacted to high-intensity vocalisations and most frequently moved towards the vocalising piglets when the maximum intensity of the isolation call was reduced.

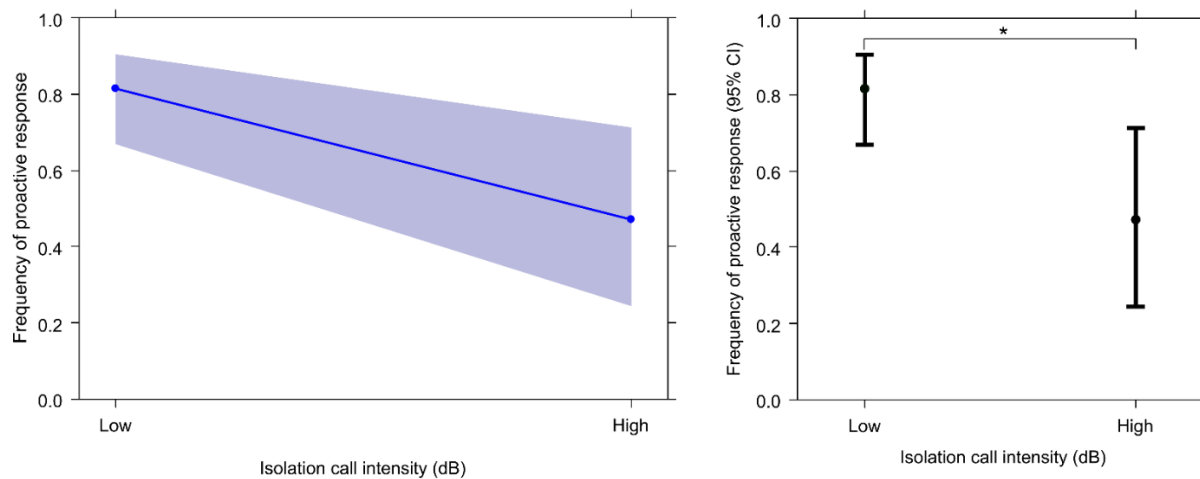


Figure 7. The effect plot (right) shows that the factor “isolation call intensity” (low/high; X axis) has a significant main effect on the target variable, that is the type of response (proactive/reactive response). The proactive response frequency (Y axis) was higher with low than with high intensity isolation calls (likelihood ratio test: $\chi^2 = 6.390$, $df = 2$, $p = 0.041$). The slope indicates the magnitude of main effect. The error bars (left) shows confidence interval (CI, black bars) around the mean (central black dot) of the frequency of proactive response (Y axis) as function of the isolation call intensity (low/high; X axis). * = $p < 0.05$

GLMM₃ was carried out on the lactating non-mothers and non-lactating females to verify whether the positive or negative relationship with the mother of the vocalising piglet (affiliation and aggression dyadic levels) would affect the probability of observing a response (presence/absence). The full model (including all fixed factors) and the null model (only including the random factor) did not significantly differ (likelihood ratio test: $\chi^2 = 1.323$; $df = 2$; $p = 0.516$). Hence, no predictor had a significant effect on the target variable.

GLMM₄ was carried out on lactating females (mothers and non-mothers of the vocalising piglet) to verify whether lactation frequency and duration would affect the response probability (presence/absence). The full model (including all fixed factors) and the null model (only including the random factor) did not significantly differ (likelihood ratio test: $\chi^2 = 0.325$, $df = 2$, $p = 0.850$). Thus, in this case also, no predictor had a significant effect on the target variable.

A2.4 Discussion

In this study, we found that (i) as it occurs in other social mammals (Ehret and Haack, 1981; Ehret, 1992; Nelson and Panksepp, 1998; Barros and Tomaz, 2002; Troisi, 2002; Newman, 2004, 2007; Bohn et al., 2006; Lingle et al., 2012; Parsons et al., 2012; Zeskind, 2013; Lingle and Riede, 2014; Del Vecchio et al., 2009; Silvers et al., 2017; Hiraoka et al., 2020), anxiety levels and response behaviour increased in tandem, both showing a peak when piglets emit the isolation call; (ii) lactating females (but not necessarily mothers) responded more to piglets in distress, in partial agreement with previous studies in mammals according to which mothers are the most responsive (Loudon and Racey, 1988); (iii) higher proactive response levels when isolation calls were characterised by low (rather than high) intensity, possibly because other acoustic features may interfere (such as amplitude and fundamental frequency Briefer et al., 2022). Indeed, taken all together, these results are in line with the adaptive role that isolation calls or cries play in mammals. For example, in *Homo sapiens*, the crying of infants has signalling functions essential for their survival (Nelson and Panksepp, 1998; Zeifman, 2001; Lingle et al., 2012). "Among non-human primates, infants of common marmosets (*Callithrix jacchus*) emit their species-specific isolation calls (called "isolation phee"), and similar acoustic signals have been described in rhesus macaques (*Macaca mulatta*) to elicit mothers' attention (Epple, 1968; Norcross and Newman, 1993; Newman, 1995) and in strepsirhines (Zimmermann, 1995). Beyond primates, isolation calls are used to possibly get attention in bats of different species (Gould, 1971; Gould et al., 1973; Gould, 1975; Habersetzer and Marimuthu, 1986; Scherrer and Wilkinson, 1993), guinea pigs (Berryman, 1976), mice (Koch and Ehret, 1989; Hudson et al., 2015), kittens (Haskins, 1977; Brown et al., 1978; Scheumann et al., 2012; Tavernier et al., 2020), reindeer (Espmark, 1971), pinnipeds (Trillmich, 1981; Insley, 1992), and cetaceans (Sayigh et al., 1990; Mishima et al., 2015). Our results are discussed in detail below.

A2.4.1. Sow Response and Anxiety Behaviours

As expected, we found increased levels of anxiety-related behaviour and increased frequency of response behaviour in the sows following the isolation calls of piglets (**Figure 4a, b**; assumptions 1a and 1b from **Table 3** confirmed). Thus, the increase in the response frequency of sows may be related to an increase in anxiety levels, which could lead to increased vigilance. In line with previous literature (Gross and Hen, 2004; Lonstein et al., 2015; Silvers et al., 2017; Breedveld et al., 2019), our results may suggest that a key role is played by increased vigilance in sows following hearing the isolation call emitted by piglets.

In social species, recognising stress signals emitted by other individuals can be highly adaptive (Preston and de Waal, 2002). Our findings could suggest that sows are able to recognise the distress of piglets and enact behavioural patterns that lead to distress reduction. This finding is in agreement with observations on other mammalian species—including rodents, domestic ungulates, and non-human and human primates—which indicate that acoustic signals emitted by immature subjects can provide distinctive and reliable indications of the offspring's emotional state to attract

the mother's attention and elicit a rapid behavioural response (Pettijohn, 1977; Grafen, 1990; Weary et al., 1996; Jovanovic et al., 2000; Špinka et al., 2002; Soltis, 2004; Mizuno et al., 2006; Scheumann et al., 2007; Rendall et al., 2009; Decety et al., 2016; Hiraoka and Nomura, 2016; de Waal and Preston, 2017; Schnaider et al., 2022). In particular, our results are in line with previous studies on domestic pigs that have shown that piglets' vocalisations may communicate their emotional state and attract the mother's attention (Weary et al., 1996; Špinka et al., 2002) and that individuals are sensitive to the internal states of others and can react to their distress (Goumon and Špinka, 2016; Reimert et al., 2013, 2015, 2017). Thus, considering our results, we can highlight that sows are able to react to the vocalisations of piglets experiencing distress when isolated and that in this species, the response of the sows to the acoustic signals may be decisive for the survival of the offspring.

Besides increased vigilance, which remains the most parsimonious explanation, sow responses might be related to affective arousal triggered by the detection of conspecifics' distress. This phenomenon—known as emotional contagion—has been observed in several mammalian species, including the domestic pig (Reimert et al., 2013). In line with this hypothesis, this phenomenon can be particularly pronounced during maternal care, because mothers are emotionally affected by the state of their offspring (Preston and de Waal, 2002). In particular, in humans, mothers may experience physiological arousal when they witness their baby crying (Zeskind and Collins, 1987; Parsons et al., 2012), and infant cries can be particularly stressful for mothers (Sapolsky et al., 2000; Fujiwara et al., 2011; Swain et al., 2011), requiring a rapid response to provide the necessary care to infants (Giardino et al., 2008; Parsons et al., 2012). Further studies are necessary to investigate this aspect by comparing the reactions of sows to emotional and non-emotional stimuli.

Therefore, the results of this study seem in line with the evolutionary importance of the ability to recognise and react to distress signals in infants in order to allow a higher probability of offspring survival.

A2.4.2. Individual, Perceptive, and Social Factors Affecting Sow Response

We expected that lactation and motherhood would enhance the sow response to piglet isolation calls. However, this expectation was not fully supported because the duration and frequency of lactation did not affect the response levels in sows (assumption 4 not confirmed), even though lactating females (not necessarily the mothers of the distressed piglets) responded more than non-lactating females (**Figure 6**, assumption 1c from **Table 3** partially confirmed). This result may be explained by the fact that sows can show high inter-individual variability in the duration and frequency of lactation (Hawe et al., 2020). However, the response of the sows under study appeared to be mainly mediated by lactation per se rather than by its duration and frequency, possibly due to circulating pro-maternal hormones. Indeed, hormones implicated in delivery, lactation, and weaning (i.e., mostly prolactin and oxytocin) lead to an increase in care behaviours (e.g., retrieval, nursing, and grooming) and the formation of mother-infant attachment (Bridges et al., 1985; Prevost et al., 2014; Brown et al., 2017; Johnson and Young, 2017). Interestingly, oxytocin may increase the salience of infant vocalisations by stimulating retrieval behaviour in mothers (Marlin et al., 2015).

We expected a higher frequency of response to piglet isolation calls by sows showing lower levels of aggression. In agreement with this assumption, we found that the level of aggression had a significant effect, with less aggressive sows showing the highest levels of responses (**Figure 5**; assumption 1d from **Table 3** confirmed). As concerns affiliation, we expected a higher frequency of response to piglet isolation calls by sows showing generally higher levels of affiliation and, more specifically, by sows sharing more affiliation with the mother of the vocalising piglet. Contrary to these assumptions, sow affiliation levels did not affect the response (assumptions 1e and 3 from **Table 3** not confirmed). Hence, affiliation did not affect the sow response either at the individual or at the dyadic level, probably because lactation, more than other social features, influenced the sow response. Moreover, it must be considered that the sows were closely related to each other—siblings or half-siblings—and this may have influenced the levels of affiliation, with individual frequencies of affiliation being quite similar across sows. Moreover, aggression can be linked to testosterone, which also in females (and not just in males) can antagonise the effect of pro-maternal hormones and increase aggressive behaviour (Archer, 1991; Witczak et al., 2018; Simpson, 2020). These aspects are in line with our results showing higher levels of response from sows that exhibited lower frequencies of aggressive behaviour.

We expected that the highest frequencies of behavioural responses by sows would be associated with more intense and longer-lasting piglet isolation calls. Contrary to this assumption, our results show that proactive response levels are higher when isolation calls are characterised by lower intensity (lowest maximum dB), whereas there are no differences in the levels of reactive responses, and the duration of piglet isolation calls did not have a significant effect (**Figure 7**, assumption 4 from **Table 3** not confirmed). In this regard, this aspect of piglets' isolation calls seems to play a key role in eliciting the approach behaviour of sows towards infants in distress. This would seem to indicate that there may be characteristics of the isolation call that allow a piglet in distress to effectively communicate its state of need and attract the sow's attention.

However, with respect to the response of sows, our results may offer interesting insights. Mothers may respond to isolation calls by regulating their own behaviour, and response behaviours are conserved among mammals, including humans. In order to explain this result, it is important to take into account that—from the point of view of social cognition—proactive responses may involve an additional cognitive step in that an uninvolved third party must first detect and process subject's distress (caused by another subject or an external event) and then approach the distressed individual. Therefore, elements of complex regulation mechanisms may be present (Zaki and Williams, 2013), as the uninvolved party takes the initiative and actively approaches the source of stress in order to possibly modify its own experience (intrinsic regulation) and/or the contacted party's experience (extrinsic regulation).

Finally, during vocal production, emotions can influence the physiological structures underlying sound production and thus change the structure of the sound itself, including the duration of the vocalisation, amplitude, fundamental frequency, and energy distribution (Briefer, 2012). Therefore, other features of isolation calls could also have an effect on the response behaviour of sows, and it would be interesting to investigate this in future studies.

A possible limitation of this study may be related to the small sample size (if we consider the absolute number of individuals) and to differences in the age of lactating and non-lactating sows, so we encourage future studies to expand and/or replicate this

investigation to possibly make inferences at the population or species level. On the other hand, this investigation was carried out under extensive, natural farming conditions, and in terms of study design, we managed to have a similar number of lactating and non-lactating (sexually mature) females exposed to the same environmental conditions in the same season (hence, the same climate), with the piglets all falling within the pre-weaning period. The perception and recognition of a state of anxiety in another individual could be further key factors, and we cannot exclude that the behavioural response observed by the sow might be the result of emotional contagion. From an adaptive point of view, all these behavioural aspects could contribute to increasing the survival probability of the offspring.

A2.4.3Conclusions

Hence, our study can add to our knowledge of how sows ecologically respond to distressed piglets. In particular, our results show increased levels of anxiety behaviour in sows, accompanied by increased responses to distressed piglets. Indeed, the higher levels of response in lactating females compared to non-mothers may be explained by the influence that maternal hormones could have on the behaviour of lactating sows by increasing their care activities and recovering/vigilance and by stimulating mother-offspring attachment (Bridges et al., 1985; Marlin et al., 2015; Brown et al., 2017; Johnson and Young, 2017; Hawe et al., 2020;). In conclusion, the results of this study highlight the possible adaptive value of sows' ability to recognise manifestations of others' anxiety and consequently react appropriately.

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Ethical Statement: This study is part of the GREENPIG project and was approved by the Bioethic committee of the University of Turin (permit n. 0615995). This study was conducted in accordance with the Italian law Dlg. 4/2014 n. 26 (based on European provisions), which regulates the use of animals in experimental and scientific research.

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A2.5 References

- Algers, B. (1993). Nursing in pigs: Communicating needs and distributing resources. *Journal of Animal Science*, 71(10), 2826–2831. <https://doi.org/10.2527/1993.71102826x>
- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49(3–4), 227–266. <https://doi.org/10.1163/156853974x00534>
- Archer, J. (1991). The influence of testosterone on human aggression. *British Journal of Psychology (London, England: 1953)*, 82(1), 1–28. <https://doi.org/10.1111/j.2044-8295.1991.tb02379.x>
- Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, 68(3), 255–278. <https://doi.org/10.1016/j.jml.2012.11.001>
- Barros, M., & Tomaz, C. (2002). Non-human primate models for investigating fear and anxiety. *Neuroscience and Biobehavioral Reviews*, 26(2), 187–201. [https://doi.org/10.1016/s0149-7634\(01\)00064-1](https://doi.org/10.1016/s0149-7634(01)00064-1)
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Baxter, E. M., Hall, S. A., Farish, M., Donbavand, J., Brims, M., Jack, M., & Lawrence, A. B. (2023). Camerlink, I. Piglets' behaviour and performance in relation to sow characteristics. *Animal*, 17.
- Berg, R. E., & Stork, D. G. (2004). *The Physics of Sound* (Vol. 9780131457898). Upper Saddle River, NJ, USA: Pearson.
- Berryman, J. C. (1976). Guinea-pig vocalizations: Their structure, causation, and function. *Zeitschrift Für Tierpsychologie*, 41(1), 80–106. <https://doi.org/10.1111/j.1439-0310.1976.tb00471.x>
- Birteeb, P. T., Tetteh, S. O., & Salifu, A. S. (2015). Growth performance and weight estimation of Large White piglets weaned at different ages. *Res. Rev. J. Vet. Sci. Technol*, 4, 15–23.
- Bohn, K. M., Moss, C. F., & Wilkinson, G. S. (2006). Correlated evolution between hearing sensitivity and social calls in bats. *Biology Letters*, 2(4), 561–564. <https://doi.org/10.1098/rsbl.2006.0501>
- Breedveld, M. C., Folkertsma, R., & Eccard, J. A. (2019). Rodent mothers increase vigilance behaviour when facing infanticide risk. *Scientific Reports*, 9(1), 12054. <https://doi.org/10.1038/s41598-019-48459-9>
- Bretz, F., Hothorn, T., & Westfall, P. (2016). *Multiple comparisons using R*. Chapman and Hall/CRC.

Bridges, R. S., DiBiase, R., Loundes, D. D., & Doherty, P. C. (1985). Prolactin stimulation of maternal behavior in female rats. *Science (New York, N.Y.)*, 227(4688), 782–784. <https://doi.org/10.1126/science.3969568>

Briefer, E. F. (2012). Vocal expression of emotions in mammals: Mechanisms of production and evidence: Vocal communication of emotions. *J. Zool.*, 288, 1–20.

Briefer, E. F., Sypherd, C. C.-R., Linhart, P., Leliveld, L. M. C., Padilla de la Torre, M., Read, E. R., ... Tallet, C. (2022). Classification of pig calls produced from birth to slaughter according to their emotional valence and context of production. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-07174-8>

Brown, K. A., Buchwald, J. S., Johnson, J. R., & Mikolich, D. J. (1978). Vocalization in the cat and kitten. *Developmental Psychobiology*, 11(6), 559–570. <https://doi.org/10.1002/dev.420110605>

Brown, R. S. E., Aoki, M., Ladyman, S. R., Phillipps, H. R., Wyatt, A., Boehm, U., & Grattan, D. R. (2017). Prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 114(40), 10779–10784. <https://doi.org/10.1073/pnas.1708025114>

Brudzynski, S. M. (2007). Ultrasonic calls of rats as indicator variables of negative or positive states: Acetylcholine-dopamine interaction and acoustic coding. *Behavioural Brain Research*, 182(2), 261–273. <https://doi.org/10.1016/j.bbr.2007.03.004>

Chaiken, M. (1992). Individual recognition of nestling distress screams by European starlings (*Sturnus vulgaris*). *Behaviour*, 120(1–2), 139–150. <https://doi.org/10.1163/156853992x00255>

Charrier, I., Mathevon, N., & Jouventin, P. (2002). How does a fur seal mother recognize the voice of her pup? An experimental study of *Arctocephalus tropicalis*. *The Journal of Experimental Biology*, 205(Pt 5), 603–612. <https://doi.org/10.1242/jeb.205.5.603>

Cordoni, G., Comin, M., Collarini, E., Robino, C., Chierto, E., & Norscia, I. (2023). Domestic pigs (*Sus scrofa*) engage in non-random post-conflict affiliation with third parties: Cognitive and functional implications. *Animal Cognition*, 26(2), 687–701. <https://doi.org/10.1007/s10071-022-01688-4>

de Waal, F. B. M., & Preston, S. D. (2017). Mammalian empathy: Behavioural manifestations and neural basis. *Nature Reviews. Neuroscience*, 18(8), 498–509. <https://doi.org/10.1038/nrn.2017.72>

Decety, J., Barta, I. B.-A., Uzefovsky, F., & Knafo-Noam, A. (2016). Empathy as a driver of prosocial behaviour: Highly conserved neurobehavioural mechanisms across species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 371(1686), 20150077. <https://doi.org/10.1098/rstb.2015.0077>

Del Vecchio, T., Walter, A., & Leary, A. (2009). Affective and physiological factors predicting maternal response to infant crying. *Infant Behav. Dev.*, 32, 117–122.

Dobson, A. J., & Barnett, A. G. (2018). *An introduction to generalized linear models* (4th ed.). <https://doi.org/10.1201/9781315182780>

Durrell, J. L., Sneddon, I. A., O'Connell, N. E., & Whitehead, H. (2004). Do pigs form preferential associations? *Applied Animal Behaviour Science*, 89(1–2), 41–52. <https://doi.org/10.1016/j.applanim.2004.05.003>

Ehret, G. (1992). Categorical perception of mouse-pup ultrasounds in the temporal domain. *Animal Behaviour*, 43(3), 409–416. [https://doi.org/10.1016/s0003-3472\(05\)80101-0](https://doi.org/10.1016/s0003-3472(05)80101-0)

Ehret, G., & Haack, B. (1981). Categorical perception of mouse pup ultrasound by lactating females. *The Science of Nature*, 68(4), 208–209. <https://doi.org/10.1007/bf01047208>

Epple, G. (1968). Comparative studies on vocalization in marmoset monkeys (*Hapalidae*). *Folia Primatologica; International Journal of Primatology*, 8(1), 1–40. <https://doi.org/10.1159/000155129>

Espmark, Y. (1971). Individual recognition by voice in reindeer mother-young relationship. Field observations and playback experiments. *Behaviour*, 40(3–4), 295–301. <https://doi.org/10.1163/156853971x00438>

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: Overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology*, 65(1), 47–55. <https://doi.org/10.1007/s00265-010-1038-5>

Fraser, D. (1975). Vocalizations of isolated piglets. I. Sources of variation and relationships among measures. *Applied Animal Ethology*, 1(4), 387–394. [https://doi.org/10.1016/0304-3762\(75\)90075-9](https://doi.org/10.1016/0304-3762(75)90075-9)

Fujiwara, T., Barr, R. G., Brant, R., & Barr, M. (2011). Infant distress at five weeks of age and caregiver frustration. *The Journal of Pediatrics*, 159(3), 425–430.e1–2. <https://doi.org/10.1016/j.jpeds.2011.02.010>

Giardino, J., Gonzalez, A., Steiner, M., & Fleming, A. S. (2008). Effects of motherhood on physiological and subjective responses to infant cries in teenage mothers: A comparison with non-mothers and adult mothers. *Hormones and Behavior*, 53(1), 149–158. <https://doi.org/10.1016/j.yhbeh.2007.09.010>

Gould, E. (1971). Studies of maternal-infant communication and development of vocalizations in the bats *Myotis* and *Eptesicus*. *Communications in Behavioral Biology*, 5, 263–313.

Gould, E. (1975). Neonatal vocalizations in bats of eight genera. *Journal of Mammalogy*, 56(1), 15–29. <https://doi.org/10.2307/1379603>

Gould, E., Woolf, N. K., & Turner, D. C. (1973). Double-note communication calls in bats: Occurrence in three families. *Journal of Mammalogy*, 54(4), 998–1001. <https://doi.org/10.2307/1379103>

Goumon, S., Illmann, G., Leszkowová, I., Dostalová, A., & Cantor, M. (2020). Dyadic affiliative preferences in a stable group of domestic pigs. *Applied Animal Behaviour Science*, 230, 105045. <https://doi.org/10.1016/j.applanim.2020.105045>

Goumon, S., & Špinka, M. (2016). Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Animal Cognition*, 19(3), 501–511. <https://doi.org/10.1007/s10071-015-0950-5>

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology*, 144(4), 517–546. [https://doi.org/10.1016/s0022-5193\(05\)80088-8](https://doi.org/10.1016/s0022-5193(05)80088-8)

Gross, C., & Hen, R. (2004). The developmental origins of anxiety. *Nature Reviews. Neuroscience*, 5(7), 545–552. <https://doi.org/10.1038/nrn1429>

Habersetzer, J., & Marimuthu, G. (1986). Ontogeny of sounds in the echolocating bat *Hipposideros speoris*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 158(2), 247–257. <https://doi.org/10.1007/bf01338568>

Hahn, M. E., & Lavooy, M. J. (2005). A review of the methods of studies on infant ultrasound production and maternal retrieval in small rodents. *Behavior Genetics*, 35(1), 31–52. <https://doi.org/10.1007/s10519-004-0854-7>

Hartig, F. (2023). DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models. *R-project.org*. Retrieved July 4, 2023, from <https://cran.r-project.org/web/packages/DHARMA/vignettes/DHARMA.html>

Haskins, R. (1977). Effect of kitten vocalizations on maternal behavior. *Journal of Comparative and Physiological Psychology*, 91(4), 830–838. <https://doi.org/10.1037/h0077362>

Hawe, S. J., Scollan, N., Gordon, A., & Magowan, E. (2020). Impact of sow lactation feed intake on the growth and suckling behavior of low and average birthweight pigs to 10 weeks of age. *Translational Animal Science*, 4(2), txaa057. <https://doi.org/10.1093/tas/txaa057>

Hiraoka, D., & Nomura, M. (2016). The influence of cognitive load on empathy and intention in response to infant crying. *Scientific Reports*, 6, 28247. <https://doi.org/10.1038/srep28247>

Hiraoka, D., Ooishi, Y., Mugitani, R., & Nomura, M. (2020). Relationship between oxytocin and maternal approach behaviors to infants' vocalizations. *Comprehensive Psychoneuroendocrinology*, 4, 100010. <https://doi.org/10.1016/j.cpnec.2020.100010>

Hudson, R., Rangassamy, M., Saldaña, A., Bánszegi, O., & Rödel, H. G. (2015). Stable individual differences in separation calls during early development in cats and mice. *Frontiers in Zoology*, 12(Suppl 1), S12. <https://doi.org/10.1186/1742-9994-12-S1-S12>

Illmann, G., Hammerschmidt, K., Špinka, M., & Tallet, C. (2013). Calling by domestic piglets during simulated crushing and isolation: A signal of need? *PloS One*, 8(12), e83529. <https://doi.org/10.1371/journal.pone.0083529>

Illmann, G., Schrader, L., Špinka, M., & Šustr, P. (2002). Acoustical mother-offspring recognition in pigs (*Sus scrofa domestica*). *Behaviour*, 139(4), 487–505. <https://doi.org/10.1163/15685390260135970>

Insley, S. J. (1992). Mother-offspring separation and acoustic stereotypy: A comparison of call morphology in two species of pinnipeds. *Behaviour*, 120(1–2), 103–122. <https://doi.org/10.1163/156853992x00237>

Insley, S. J., Phillips, A. V., & Charrier, I. (2003). A review of social recognition in pinnipeds. *Aquatic Mammals*, 29(2), 181–201. <https://doi.org/10.1578/016754203101024149>

Johnson, Z. V., & Young, L. J. (2017). Oxytocin and vasopressin neural networks: Implications for social behavioral diversity and translational neuroscience. *Neuroscience and Biobehavioral Reviews*, 76(Pt A), 87–98. <https://doi.org/10.1016/j.neubiorev.2017.01.034>

Jovanovic, T., Megna, N. L., & Maestripieri, D. (2000). Early maternal recognition of offspring vocalizations in rhesus macaques (*Macaca mulatta*). *Primates; Journal of Primatology*, 41(4), 421–428. <https://doi.org/10.1007/BF02557653>

Koch, M., & Ehret, G. (1989). Estradiol and parental experience, but not prolactin are necessary for ultrasound recognition and pup-retrieving in the mouse. *Physiology & Behavior*, 45(4), 771–776. [https://doi.org/10.1016/0031-9384\(89\)90293-x](https://doi.org/10.1016/0031-9384(89)90293-x)

Lester, B. M., & La Gasse, L. L. (2008). *Encyclopedia of Infant and Early Childhood Development* (Haith, M. M. Ed.). San Diego, CA, USA: Academic Press.

Lingle, S., Rendall, D., & Pellis, S. M. (2007). Altruism and recognition in the antipredator defence of deer: 1. Species and individual variation in fawn distress calls. *Animal Behaviour*, 73(5), 897–905. <https://doi.org/10.1016/j.anbehav.2006.11.003>

Lingle, S., & Riede, T. (2014). Deer mothers are sensitive to infant distress vocalizations of diverse mammalian species. *The American Naturalist*, 184(4), 510–522. <https://doi.org/10.1086/677677>

Lingle, S., Wyman, M. T., Kotrba, R., Teichroeb, L. J., & Romanow, C. A. (2012). What makes a cry a cry? A review of infant distress vocalizations. *Current Zoology*, 58(5), 698–726. <https://doi.org/10.1093/czoolo/58.5.698>

Lonstein, J. S., Lévy, F., & Fleming, A. S. (2015). Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. *Hormones and Behavior*, 73, 156–185. <https://doi.org/10.1016/j.yhbeh.2015.06.011>

Loudon, A. S. I., & Racey, P. (1988). *Reproductive Energetics in Mammals*. Clarendon Press.

Løvendahl, P., Damgaard, L. H., Nielsen, B. L., Thodberg, K., Su, G., & Rydhmer, L. (2005). Aggressive behaviour of sows at mixing and maternal behaviour are heritable and genetically correlated traits. *Livestock Production Science*, 93(1), 73–85. <https://doi.org/10.1016/j.livprodsci.2004.11.008>

Manteuffel, G., Puppe, B., & Schön, P. C. (2004). Vocalization of farm animals as a measure of welfare. *Applied Animal Behaviour Science*, 88(1–2), 163–182. <https://doi.org/10.1016/j.applanim.2004.02.012>

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, 520(7548), 499–504. <https://doi.org/10.1038/nature14402>

Mishima, Y., Morisaka, T., Itoh, M., Matsuo, I., Sakaguchi, A., & Miyamoto, Y. (2015). Individuality embedded in the isolation calls of captive beluga whales (*Delphinapterus leucas*). *Zoological Letters*, 1(1), 27. <https://doi.org/10.1186/s40851-015-0028-x>

Mizuno, Y., Takeshita, H., & Matsuzawa, T. (2006). Behavior of infant chimpanzees during the night in the first 4 months of life: Smiling and suckling in relation to behavioral state. *Infancy: The Official Journal of the International Society on Infant Studies*, 9(2), 221–240. https://doi.org/10.1207/s15327078in0902_7

Mutua, F., Dewey, C., Arimi, S., Schelling, E., & Ogara, W. (2011). Prediction of live body weight using length and girth measurements for pigs in rural Western Kenya. *Journal of Swine Health and Production*, 19(1), 26–33. <https://doi.org/10.54846/jshap/651>

Nelson, E. E., & Panksepp, J. (1998). Brain substrates of infant-mother attachment: Contributions of opioids, oxytocin, and norepinephrine. *Neuroscience and Biobehavioral Reviews*, 22(3), 437–452. [https://doi.org/10.1016/s0149-7634\(97\)00052-3](https://doi.org/10.1016/s0149-7634(97)00052-3)

Newberry, R. C., & Wood-Gush, D. G. M. (1986). Social relationships of piglets in a semi-natural environment. *Animal Behaviour*, 34(5), 1311–1318. [https://doi.org/10.1016/s0003-3472\(86\)80202-0](https://doi.org/10.1016/s0003-3472(86)80202-0)

Newman, J. D. (1995). Vocal ontogeny in macaques and marmosets: Convergent and divergent lines of development. In *Current Topics in Primate Vocal Communication* (pp. 73–97). Springer US.

Newman, J. D. (2004). The primate isolation call: A comparison with precocial birds and non-primate mammals. In *Comparative Vertebrate Cognition* (pp. 171–187). Springer US.

Newman, J. D. (2007). Neural circuits underlying crying and cry responding in mammals. *Behavioural Brain Research*, 182(2), 155–165. <https://doi.org/10.1016/j.bbr.2007.02.011>

Norcross, J. L., & Newman, J. D. (1993). Context and gender-specific differences in the acoustic structure of common marmoset (*Callithrix jacchus*) phee calls. *American Journal of Primatology*, 30(1), 37–54. <https://doi.org/10.1002/ajp.1350300104>

Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>

Ocepek, M., & Andersen, I. L. (2017). What makes a good mother? Maternal behavioural traits important for piglet survival. *Applied Animal Behaviour Science*, 193, 29–36. <https://doi.org/10.1016/j.applanim.2017.03.010>

Ocepek, M., & Andersen, I. L. (2018). Sow communication with piglets while being active is a good predictor of maternal skills, piglet survival and litter quality in three different breeds of domestic pigs (*Sus scrofa domesticus*). *PloS One*, 13(11), e0206128. <https://doi.org/10.1371/journal.pone.0206128>

Parsons, C. E., Young, K. S., Parsons, E., Stein, A., & Kringelbach, M. L. (2012). Listening to infant distress vocalizations enhances effortful motor performance: Infant cries improve adults' motor performance. *Acta Paediatrica*, 101, e189–e191.

Pettijohn, T. F. (1977). Reaction of parents to recorded infant guinea pig distress vocalization. *Behavioral Biology*, 21(3), 438–442. [https://doi.org/10.1016/s0091-6773\(77\)90281-4](https://doi.org/10.1016/s0091-6773(77)90281-4)

Pierce, A. D., & Beyer, R. T. (1990). *Acoustics: An introduction to its physical principles and applications*. The Journal of the Acoustical Society of America, 87(4), 1826–1827. <https://doi.org/10.1121/1.399390>

Preston, S. D., & de Waal, F. B. M. (2002). Empathy: Its ultimate and proximate bases. *The Behavioral and Brain Sciences*, 25(1), 1–20; discussion 20–71. <https://doi.org/10.1017/s0140525x02000018>

Prevost, M., Zelkowitz, P., Tulandi, T., Hayton, B., Feeley, N., Carter, C. S., ... Gold, I. (2014). Oxytocin in pregnancy and the postpartum: Relations to labor and its management. *Frontiers in Public Health*, 2, 1. <https://doi.org/10.3389/fpubh.2014.00001>

R Core Team. (2023). The R project for statistical computing. Retrieved July 4, 2023, from R-project.org website: <http://www.R-project.org/>

Reiland, S. (1978). Growth and skeletal development of the pig. *Acta Radiologica Supplementum*, 358, 15–22.

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2013). Indicators of positive and negative emotions and emotional contagion in pigs. *Physiology & Behavior*, 109, 42–50. <https://doi.org/10.1016/j.physbeh.2012.11.002>

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2015). Emotions on the loose: Emotional contagion and the role of oxytocin in pigs. *Animal Cognition*, 18(2), 517–532. <https://doi.org/10.1007/s10071-014-0820-6>

Reimert, I., Fong, S., Rodenburg, T. B., & Bolhuis, J. E. (2017). Emotional states and emotional contagion in pigs after exposure to a positive and negative treatment. *Applied Animal Behaviour Science*, 193, 37–42. <https://doi.org/10.1016/j.applanim.2017.03.009>

Rendall, D., Notman, H., & Owren, M. J. (2009). Asymmetries in the individual distinctiveness and maternal recognition of infant contact calls and distress screams in baboons. *The Journal of the Acoustical Society of America*, 125(3), 1792–1805. <https://doi.org/10.1121/1.3068453>

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21(1), 55–89. <https://doi.org/10.1210/edrv.21.1.0389>

Sayigh, L., Tyack, P., Wells, R., & Scott, M. (1990). Signature whistles of free-ranging bottlenose dolphins *Tursiops truncatus*: Stability and mother-offspring comparisons. *Behavioral Ecology and Sociobiology*, 26(4). <https://doi.org/10.1007/bf00178318>

Scherrer, J. A., & Wilkinson, G. S. (1993). Evening bat isolation calls provide evidence for heritable signatures. *Animal Behaviour*, 46(5), 847–860. <https://doi.org/10.1006/anbe.1993.1270>

Scheumann, M., Roser, A.-E., Konerding, W., Bleich, E., Hedrich, H.-J., & Zimmermann, E. (2012). Vocal correlates of sender-identity and arousal in the isolation calls of domestic kitten (*Felis silvestris catus*). *Frontiers in Zoology*, 9(1), 36. <https://doi.org/10.1186/1742-9994-9-36>

Scheumann, M., Zimmermann, E., & Deichsel, G. (2007). Context-specific calls signal infants' needs in a strepsirrhine primate, the gray mouse lemur (*Microcebus murinus*). *Developmental Psychobiology*, 49(7), 708–718. <https://doi.org/10.1002/dev.20234>

Schnaider, M. A., Heidemann, M. S., Silva, A. H. P., Taconeli, C. A., & Molento, C. F. M. (2022). Vocalization and other behaviors as indicators of emotional valence: The case of cow-calf separation and reunion in beef cattle. *Journal of Veterinary Behavior: Clinical Applications and Research: Official Journal of: Australian Veterinary Behaviour Interest Group, International Working Dog Breeding Association*, 49, 28–35. <https://doi.org/10.1016/j.jveb.2021.11.011>

Schon, P.-C., Gromyko, T., Manteuffel, G., & Puppe, B. (1999). Common features and individual differences in nurse grunting of domestic pigs (*Sus scrofa*): A multi-parametric analysis. *Behaviour*, 136(1), 49–66. <https://doi.org/10.1163/156853999500668>

Schön, P.-C., Puppe, B., & Manteuffel, G. (1998). A sound analysis system based on Labview® applied to the analysis of suckling grunts of domestic pig *Sus scrofa*. *Bioacoustics*, 9, 119–133.

Signoret, J. P., Baldwin, B. A., Fraser, D., & Hafez, E. S. (1975). The behaviour of swine. In *Behaviour of domestic animals* (pp. 295–329). London, UK: Baillière Tindall.

Silvers, J. A., Goff, B., Gabard-Durnam, L. J., Gee, D. G., Fareri, D. S., Caldera, C., & Tottenham, N. (2017). Vigilance, the amygdala, and anxiety in youths with a history of institutional care. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, 2(6), 493–501. <https://doi.org/10.1016/j.bpsc.2017.03.016>

Simpson, K. (2020). The role of testosterone in aggression. *McGill Journal of Medicine: MJM: An International Forum for the Advancement of Medical Sciences by Students*, 6(1). <https://doi.org/10.26443/mjm.v6i1.559>

Soltis, J. (2004). The signal functions of early infant crying. *The Behavioral and Brain Sciences*, 27(4), 443–458. <https://doi.org/10.1017/s0140525x0400010x>

Špinka, M., Stěhulová, I., Zachařová, J., Maletínská, J., & Illmann, G. (2002). Nursing behaviour and nursing vocalisations in domestic sows: Repeatability and relationship with maternal investment. *Behaviour*, 139(8), 1077–1097. <https://doi.org/10.1163/15685390260337912>

- Swain, J. E., Kim, P., & Ho, S. S. (2011). Neuroendocrinology of parental response to baby-cry: Neuroendocrinology of parental response to baby-cry. *Journal of Neuroendocrinology*, 23(11), 1036–1041. <https://doi.org/10.1111/j.1365-2826.2011.02212.x>
- Tavernier, C., Ahmed, S., Houpt, K. A., & Yeon, S. C. (2020). Feline vocal communication. *Journal of Veterinary Science*, 21(1), e18. <https://doi.org/10.4142/jvs.2020.21.e18>
- Torriani, M. V. G., Vannoni, E., & McElligott, A. G. (2006). Mother-young recognition in an ungulate hider species: A unidirectional process. *The American Naturalist*, 168(3), 412–420. <https://doi.org/10.1086/506971>
- Trillmich, F. (1981). Mutual mother-pup recognition in Galápagos fur seals and sea lions: Cues used and functional significance. *Behaviour*, 78(1–2), 21–42. <https://doi.org/10.1163/156853981x00248>
- Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress (Amsterdam, Netherlands)*, 5(1), 47–54. <https://doi.org/10.1080/102538902900012378>
- Weary, D. M., & Fraser, D. (1995). Calling by domestic piglets: Reliable signals of need? *Animal Behaviour*, 50(4), 1047–1055. [https://doi.org/10.1016/0003-3472\(95\)80105-7](https://doi.org/10.1016/0003-3472(95)80105-7)
- Weary, D. M., Lawson, G. L., & Thompson, B. K. (1996). Sows show stronger responses to isolation calls of piglets associated with greater levels of piglet need. *Animal Behaviour*, 52(6), 1247–1253. <https://doi.org/10.1006/anbe.1996.0272>
- Weary, D. M., Ross, S., & Fraser, D. (1997). Vocalizations by isolated piglets: A reliable indicator of piglet need directed towards the sow. *Applied Animal Behaviour Science*, 53(4), 249–257. [https://doi.org/10.1016/s0168-1591\(96\)01173-2](https://doi.org/10.1016/s0168-1591(96)01173-2)
- Witczak, L. R., Ferrer, E., & Bales, K. L. (2018). Effects of aggressive temperament on endogenous oxytocin levels in adult titi monkeys. *American Journal of Primatology*, 80(10), e22907. <https://doi.org/10.1002/ajp.22907>
- Zaki, J., & Williams, W. C. (2013). Interpersonal emotion regulation. *Emotion (Washington, D.C.)*, 13(5), 803–810. <https://doi.org/10.1037/a0033839>
- Zeifman, D. M. (2001). An ethological analysis of human infant crying: Answering Tinbergen's four questions. *Developmental Psychobiology*, 39(4), 265–285. <https://doi.org/10.1002/dev.1005>
- Zeskind, P. S., & Collins, V. (1987). Pitch of infant crying and caregiver responses in a natural setting. *Infant Behavior & Development*, 10(4), 501–504. [https://doi.org/10.1016/0163-6383\(87\)90046-4](https://doi.org/10.1016/0163-6383(87)90046-4)
- Zeskind, P. S. E., Schmidt, S., & Zimmerman, E. (2013). Evolution of emotional communication: From sounds in nonhuman mammals to speech and music in man. In *Series in Affective Science* (pp. 155–172). Oxford, UK: Oxford University Press.

Zimmermann, E. (1995). Loud calls in nocturnal prosimians: Structure, evolution, and ontogeny. In *Current Topics in Primate Vocal Communication* (pp. 47–72). Boston, MA: Springer US.

About animal welfare

The concept of animal welfare has evolved over time, reflecting changes in values, beliefs, and advancements in scientific understanding of animals. There is still no unanimous consensus on what exactly constitutes animal welfare (Reimert et al., 2023). In general, it has historically been conceived as the mere absence of suffering, a concept that, while fundamental as a starting point, is incomplete when considered in isolation.

A key reference in this regard is the Five Freedoms (FAWC, 1992), a conceptual framework that has had a significant impact on international thinking on animal welfare. These freedoms – from hunger and thirst, from discomfort, from pain, from fear, and the freedom to express natural behaviours – have guided the definition of minimum welfare standards for years. However, as noted by Mellor (2016), over time, some interpretations have transformed these freedoms into absolute rights, even though they were originally conceived as goals to be pursued “as far as possible”. Furthermore, with the advancement of neuroscientific and behavioural research, it has become clear that negative states, being integral to survival processes, cannot be entirely eliminated, nor does their absence necessarily guarantee positive experiences.

A significant shift in this approach is represented by the Five Domains Model, initially proposed by Mellor and Reid (1994) and updated over time to its latest version (Mellor, 2020). This model consists of five interconnected domains: Nutrition, Physical Environment, Health, Behavioural Interactions, and Mental State. The first four domains focus on factors that generate positive or negative subjective experiences (affects), which are overall assessed in the fifth domain, the mental state. A central distinction in the model is between survival-critical affects – linked to the maintenance of homeostasis – and situation-related affects, associated with the animal’s conscious interaction with the environment, conspecifics, and, more recently, humans. The latter reflects the animal’s ability to make behavioural choices based on its needs, experiences, and environments, indicating a form of autonomy in its responses to external stimuli (Mellor and Beausoleil, 2015; Mellor, 2020).

In light of these considerations, the present work adopts an operational definition of animal welfare centred on the cumulative balance between positive and negative experiences, moving beyond the traditional view focused exclusively on the prevention of pain or stress. In other words, an animal enjoys a high level of welfare when the sum of positive experiences – including the expression of natural behaviours, meaningful social interactions, and access to enriching environmental stimuli – outweighs negative events (Boissy et al., 2007; Edgar et al., 2013). It is essential to recognise that attempts to eliminate all stressors might reduce both environmental stimulation and social support exchanges, which are crucial for promoting individual resilience to stress and maintaining the physical and psychological health of animals (Špinka, 2006; Ozbay et al., 2007; Rault, 2012). Providing farm animals with the opportunity to express their

natural behavioural repertoire is, in this sense, a way to meet fundamental needs and promote positive adaptations (Špinka, 2006). Environmental conditions and intrinsic factors (such as health, resilience, and personality) do not represent elements of welfare in themselves but act as factors that influence the overall balance of positive and negative experiences, affecting affective states and, consequently, the animal's welfare (Webb et al., 2019). Reimert et al. (2023) suggest that animal welfare can be conceptualised as a balance of positive and negative experiences over time, emphasising how affective states may change throughout the animal's life.

Another element of reflection is the type of farming system. Traditionally, research has focused primarily on welfare problems associated with intensive systems, while extensive systems have received less attention (Temple and Manteca, 2020). It is true that the latter offer various advantages, especially in the behavioural domain, allowing greater freedom of movement and expression of natural motivations. However, they are not without their own challenges. In particular, as highlighted by Villalba et al. (2016), animals are not always raised in environments compatible with their evolutionary adaptations, and the unpredictability of environmental factors – coupled with management practices – can lead to situations where there is a misalignment between the ecological characteristics of the environment and the animal's needs.

The ethological approach has provided fundamental tools to better understand these dynamics, enabling the identification of key behaviours that can indicate welfare or discomfort (Dawkins, 2003; Orihuela, 2021). In particular, the observation of affiliative behaviours, coping strategies, and the quality of social interactions allows for a more accurate assessment of psychological welfare. It has been highlighted that positive social interactions, such as physical contact and grooming, can play an important role in affective regulation processes, helping to alleviate anxiety and promote psychological well-being (Boissy et al., 2007; Norscia et al., 2021). Therefore, through the analysis of natural behaviours and social interactions, it is possible to gain a deeper understanding of animals' affective experiences, going beyond simple physical indicators of distress. This perspective allows not only for the identification of signs of suffering but also for promoting strategies that foster psychological and social well-being, thus improving the quality of life for animals in farming environments.

References

- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, 92, 375–397. <https://doi.org/10.1016/j.physbeh.2007.02.003>
- Dawkins, M. S. (2003). Behaviour as a tool in the assessment of animal welfare. *Zoology (Jena, Germany)*, 106(4), 383–387. <https://doi.org/10.1078/0944-2006-00122>
- Edgar, J. L., Mullan, S. M., Pritchard, J. C., McFarlane, U. J., & Main, D. C. (2013). Towards a 'good life' for farm animals: Development of a resource tier framework to achieve positive welfare for laying hens. *Animals*, 3, 584–605. <https://doi.org/10.3390/ani3030584>
- FAWC. (1992). FAWC updates the five freedoms. *Veterinary Record*, 131, 357–358.
- Mellor, D. J., & Beausoleil, N. J. (2015). Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. *Animal Welfare (South Mimms, England)*, 24(3), 241–253. <https://doi.org/10.7120/09627286.24.3.241>
- Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 Five Domains Model: Including human-animal interactions in assessments of animal welfare. *Animals: An Open Access Journal from MDPI*, 10(10), 1870. doi:10.3390/ani10101870
- Mellor, D. J., & Reid, C. S. W. (1994). Concepts of animal well-being and predicting the impact of procedures on experimental animals. In *Improving the well-being of animals in the research environment* (pp. 3–18). Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART)
- Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>
- Orihuela, A. (2021). Review: Management of livestock behavior to improve welfare and production. *Animal: An International Journal of Animal Bioscience*, 15(Suppl 1), 100290. <https://doi.org/10.1016/j.animal.2021.100290>
- Rault, J.-L. (2012). Friends with benefits: Social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science*, 136(1), 1–14. <https://doi.org/10.1016/j.applanim.2011.10.002>
- Reimert, I., Webb, L. E., van Marwijk, M. A., & Bolhuis, J. E. (2023). Review: Towards an integrated concept of animal welfare. *Animal: An International Journal of Animal Bioscience*, 17 Suppl 4(100838), 100838. doi:10.1016/j.animal.2023.100838
- Špinka, M. (2006). How important is natural behaviour in animal farming systems? *Applied Animal Behaviour Science*, 100(2), 117–128. <https://doi.org/10.1016/j.applanim.2006.04.006>

Temple, D., & Manteca, X. (2020). Animal welfare in extensive production systems is still an area of concern. *Frontiers in Sustainable Food Systems*, 4. <https://doi.org/10.3389/fsufs.2020.545902>

Troisi, A. (2002). Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. *Stress*, 5, 47–54. <https://doi.org/10.1080/102538902900012378>

Villalba, J. J., Manteca, X., Vercoe, P. E., Maloney, S. K., & Blache, D. (2016). Integrating nutrition and animal welfare in extensive systems. In *Animal Welfare* (pp. 135–163). Springer International Publishing.

Webb, L. E., Veenhoven, R., Harfeld, J. L., & Jensen, M. B. (2019). What is animal happiness?: What is animal happiness? *Annals of the New York Academy of Sciences*, 1438(1), 62–76. doi:10.1111/nyas.13983

Glossary - Post-Conflict Strategies

Opponent: is an individual directly involved in a conflict. The "victim" is the individual who suffers the aggression, while the "aggressor" is the individual who initiates or perpetuates the aggression. The conflict between the victim and the aggressor can be resolved through reconciliation, which is defined as the first affiliative contact exchanged between the two opponents right after the end of the aggression (de Waal and van Roosmaleen, 1979).

Third Party: A third party is an individual external to the conflict who can be involved in triadic affiliation with the victim or the aggressor. "Triadic affiliation" is defined as the first affiliative contact exchanged between one (or both) of the opponents and an uninvolved third party (Romero et al., 2009, 2011).

Bystander: A bystander is an individual who observes a conflict without actively participating. Bystanders can be involved in quadratic affiliation, where interactions between them may be part of the conflict resolution process. Quadratic affiliation is more precisely defined as the first affiliative contact exchanged between bystanders in the first minutes after a conflict (Judge and Mullen, 2005; De Marco et al., 2010; Daniel and Alves, 2015).

References

- Daniel, J. R., & Alves, R. L. (2015). Postconflict affiliation among bystanders in a captive group of Japanese macaques (*Macaca fuscata*). *International Journal of Primatology*, 36(2), 259–268. <https://doi.org/10.1007/s10764-015-9822-8>
- De Marco, M., Cozzolino, A., Dessì-Fulgheri, R., & Thierry, F. (2010). Conflicts induce affiliative interactions among bystanders in a tolerant species of macaque (*Macaca tonkeana*). *Animal Behaviour*, 80(2), 197–203.
- de Waal, F. B. M., & van Roosmalen, A. (1979). Reconciliation and consolation among chimpanzees. *Behavioral Ecology and Sociobiology*, 5(1), 55–66. <https://doi.org/10.1007/bf00302695>
- Romero, T., Castellanos, M. A., & de Waal, F. B. M. (2011). Post-conflict affiliation by chimpanzees with aggressors: Other-oriented versus selfish political strategy. *PloS One*, 6(7), e22173. <https://doi.org/10.1371/journal.pone.0022173>
- Romero, T., Colmenares, F., & Aureli, F. (2009). Testing the function of reconciliation and third-party affiliation for aggressors in hamadryas baboons (*Papio hamadryas hamadryas*). *American Journal of Primatology*, 71(1), 60–69. <https://doi.org/10.1002/ajp.20619>
- Judge, P. G., & Mullen, S. H. (2005). Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, 69(6), 1345–1355. <https://doi.org/10.1016/j.anbehav.2004.08.016>

Ethogram

Category	Behavioural pattern	Description
Anxiety-related behaviours	Body Scratching/rubbing	A pig uses its legs or a substrate to rub part of its body
	Vacuum-chewing	A pig chews with empty mouth
	Head/Body shaking	A pig vigorously shakes its head and/or body
	Yawning	A pig performs deep, long inhalation with open mouth
Aggressive behaviours	Aggressive lifting	A pig attempts to displace a fellow by lifting or levering it with snout or head
	Aggressive biting	A pig opens its mouth and closes its teeth tight on a fellow's small piece of flesh, including tail
	Aggressive mounting	A pig forces a fellow to move away by rising upon its rear
	Aggressive kicking	A pig projects one or both hind limbs towards a fellow, striking it
	Aggressive pushing	A pig presses its head, neck, shoulder or body against a fellow thus causing its moving
	Aggressive chasing	A pig pursues a fellow, which flees
	Aggressive head-knocking	A pig lurches or jerks its head hitting a fellow
	Aggressive chase	A pig pursues another pig (for more than a three body-lengths distance)
	Fighting	Two pigs mutually push one another in a head to head orientation. The pattern can involve body-to-body rotation and/or aggressive mounting, lifting, biting, attempt biting, kicking, chasing, pushing, head knocking, high pitched vocalisation, with no interruption lasting more than 10s
Affiliative behaviours	Rest in contact	Two pigs sit or lay in contact with one another
	Social touching	A pig touches a fellow with a paw or other body parts, except nose/head
	Nose-nose contact	A pig touches with its nose the nose of a fellow
	Nose-body contact	A pig touches/pushes with its nose a body part of a fellow (excluding nose)
	Head-over	A pig puts its head above the back of a fellow; rest in contact or body contact can after occur
	Feeding	A pig feeds in the trough or on provisioned food at feeding spots

Nutrition related behaviours	Drinking	Intake of water (with swallowing)
Explorative behaviours	Foraging	A pig sniffs, touches, scrapes, nibbles digs things and explores around the floor or roots floor or other things above the floor level.
	Browsing	A pig feeds from plant leaves, bark, and green
	Grazing	A pig feeds from grass
Invasive excavation behaviours	Rooting	A pig digs the ground with its snout and eats from roots and other underground material (or vacuum rooting)
	Digging/Pawing	A pig digs the ground with its paw and eats from roots and other underground material (or vacuum digging)
Play behaviour	Flopping	A piglet drops to the ground from the upright position to a sitting or lying position. There is no contact with an object or another individual that could cause the change in position.
	Head tossing	A piglet swings its head from one side to another
	Hopping	A piglet has either its two front feet or all four feet off the ground at one time through a upwards jumping movement. The piglet continues facing the same original direction for the whole of the behaviour
	Leg spreading	A piglet spreads its fore and hind limbs and it moves quickly from side to side
	Nudging	A piglet uses its snout to touch and push another piglet's body (excluding nose-nose contact) without causing the displacement of the contacted piglet
	Playing with object	A piglet manipulates an item or securely holds it in its mouth, shaking it or carrying it around
	Pivoting	A piglet twirls its body on the horizontal plane by a minimum of 90°
	Scampering	A piglet performs two or more forward directed hops in rapid succession
	Kneeling	A piglet goes down on its forelimb knees

References

- Brown, S. M., Peters, R., Nevison, I. M., & Lawrence, A. B. (2018). Playful pigs: Evidence of consistency and change in play depending on litter and developmental stage. *Applied Animal Behaviour Science*, 198, 36–43. <https://doi.org/10.1016/j.applanim.2017.09.018>
- Chaloupková, H., Illmann, G., Bartoš, L., & Špinka, M. (2007). The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Applied Animal Behaviour Science*, 103, 25–34. <https://doi.org/10.1016/j.applanim.2006.04.020>
- Cordoni, G., Gioia, M., Demuru, E., & Norscia, I. (2021). The dark side of play: Play fighting as a substitute for real fighting in domestic pigs, *Sus scrofa*. *Animal Behaviour*, 175, 21–31. <https://doi.org/10.1016/j.anbehav.2021.02.016>
- Donaldson, T. M., Newberry, R. C., Špinka, M., & Cloutier, S. (2002). Effects of early play experience on play behaviour of piglets after weaning. *Applied Animal Behaviour Science*, 79(3), 221–231. [https://doi.org/10.1016/s0168-1591\(02\)00138-7](https://doi.org/10.1016/s0168-1591(02)00138-7)
- Horback, K. (2014). Nosing around: Play in pigs. *Animal Behavior and Cognition*, 2(2), 186. <https://doi.org/10.12966/abc.05.08.2014>
- Jensen, P. (Ed.). (2002). *The ethology of domestic animals: An introductory text*. CABI Publishing.
- Newberry, R. C., Wood-Gush, D. G., & Hall, J. W. (1988). Playful behaviour of piglets. *Behavioural Processes*, 17(3), 205–216. [https://doi.org/10.1016/0376-6357\(88\)90004-6](https://doi.org/10.1016/0376-6357(88)90004-6)
- Norscia, I., Collarini, E., & Cordoni, G. (2021). Anxiety behavior in pigs (*Sus scrofa*) decreases through affiliation and may anticipate threat. *Frontiers in Veterinary Science*, 8, 630164. <https://doi.org/10.3389/fvets.2021.630164>
- Rauw, W. M. (2013). A note on the consistency of a behavioral play marker in piglets. *Journal of Animal Science and Biotechnology*, 4(1), 33. <https://doi.org/10.1186/2049-1891-4-33>
- Špinka, M. (2017). Behaviour of pigs. In P. Jensen (Ed.), *The Ethology of Domestic Animals: An Introductory Text* (3rd ed., pp. 214–227). CAB International.