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THE DARK SIDE OF PLAY: PLAY FIGHTING AS A SUBSTITUTE FOR REAL FIGHTING IN DOMESTIC PIGS (*Sus scrofa*)

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Play, especially in its social form, is a behaviour that can serve different functions depending on the species, social and physical environment, dominance relationships and players' individual features, such as age, sex, hierarchical rank, physical strength and body size (Burghardt, 2005; Fagen, 1981; Norscia & Palagi, 2011; Palagi, 2011; Pellis, Pellis & Bell, 2010a). Play is considered a rewarding activity (Trezza, Baarendse, & Vanderschuren, 2010; Vanderschuren, Achterberg, & Trezza, 2016) that has a fundamental role in favouring the development of physical, cognitive, and socio-emotional abilities and improving behavioural flexibility. Thanks to this, individuals adapt under changeable and unpredictable circumstances (Burghardt, 2010; Pellis & Pellis, 2009; Špinka, Newberry, & Bekoff, 2001). Different from other behavioural systems (e.g. aggressive, foraging, reproductive), play involves and re-organizes behaviours that are typical of a specific system or, in some lineages, it can be a by-product of the interaction of diverse systems (Pellis, Pellis, Pelletier, & Leca, 2019).

Play fighting - also named Rough-&-Tumble play - involves prolonged physical contacts between partners and it is composed (as play, in general) by motor patterns that are repeated, exaggerated, redundant and unordered (Burghardt, 2005). In play fighting, body contact is not usually associated with injuries and subjects do not protect a resource or their social status (Smith, 1997). As for communication, play context-specific movements, facial expressions and vocalizations are used to communicate the playful intent and to maintain a playful mood between players (Bekoff, 1995, 2001; Palagi et al., 2016a; Smith 1997). Moreover, an affinitive interaction often follows a play fighting session (Smith, 1997).

Across species, there is a remarkable variation in origin and maintenance of play fighting, which can occur in different forms and have different functions, in support of the idea that different behavioural systems are involved (Pellis & Pellis, 2017; Pellis et al., 2019). For example, in young and yearling yellow-bellied marmots (*Marmota flaviventris*), early social play may predict later dominance relationships between group members (Blumstein, Chung, & Smith, 2013). Conversely, in wild meerkats (*Suricata suricatta*), play fighting does not seem to either improve individual

combat skills or favour success in real fights (Sharpe, 2005). In other cases, different combinations of play fighting with potentially different functions are present. For example, in grey mouse lemurs (*Microcebus murinus*), about 80% of play fighting sessions are performed whilst competing to groom or mount one another (Pellis & Pellis, 2018). This process may lead, in the long run, to improve social bonding and emotion regulation between subjects and, at the same time, physically train them for future competitive interactions (Byers & Walker, 1995; Pellis & Iwaniuk, 2000 Pellis, Pellis, & Himmler, 2014; Vanderschuren & Trezza, 2014). Hence, play fighting may include competition for affinitive or sexual contact, and also mimic aggression. In a way, play fighting represents a paradox because it can enhance affiliation via playful competitive interactions and real competition via cooperative affiliation.

Here, we aim to explore the possible function of play fighting in domestic pigs (*Sus scrofa*), a species characterized by complex cognition, psychology, and sociality (Camerlink, Farish, D'Eath, Arnott, & Turner, 2018; Marino & Colvin, 2015; Norscia, Coco, Robino, Chierto, Cordoni, in press; Weller, Camerlink, Turner, Farish, & Arnott, 2019).

As many other domesticated species, the domestic pig (*Sus scrofa*) also shows the so called 'domestication syndrome' (*sensu* Wilkins, Wrangham, & Fitch, 2014). In this respect, the domestic pig is characterized by rapid growth, high reproductive rate, precocious physical and behavioural development, coordinated locomotion and adult behavioural elements since their first days of life (D'Eath & Turner, 2009; Jensen, 1988, 2002).Within a few minutes after birth, piglets begin to compete with each other to reach the most productive teats, which are located at the front (Blackshaw, Swain, Blackshaw, Thomas, & Gillies, 1997; D'Eath & Turner, 2009; Schmitt, Baxter, Boyle, & O'Driscoll, 2018). Usually, during these conflicts, future dominance relationships between siblings are determined with heavier individuals winning over lighter ones (Horback, 2014; Ruis, Brake, van de Burgwal, de Jong, Blokhuis, & Koolhaas, 2000). In natural or semi-natural conditions, piglets of about 3-7 days of age start to expand their social network by interacting at first with siblings and then with unfamiliar peers (D'Eath & Turner, 2009; Jensen, 2002).

As it occurs for immature individuals of many other species (Fagen, 1981), play is the predominant type of social interaction between piglets (Camerlink, et al., 2018; D'Eath & Turner, 2009; Newberry, Wood-Gush, & Hall, 1988; Ŝpinka, 2017). From early phases of life, piglets engage in vigorous play fighting sessions that are extremely rare in adulthood (Brown, Peters, Nevison, & Lawrence, 2018; Horback, 2014; Newberry et al., 1988). During play fighting, piglets wrestle head-to-head, hit at each other's flanks and bite the partner (Brown et al., 2018; Chaloupková, Illmann, Bartoš, & Špinka, 2007; Horback, 2014; Newberry et al., 1988; Pellis & Pellis, 2016; Šilerová, Špinka, Šárová, & Algers, 2010). In pigs, the patterns used during play fighting seem to be drawn from the aggressive behavioural system. As a matter of fact, such patterns are the same as those used during aggressive encounters by both young unacquainted piglets (Rushen & Pajor, 1987) and adult pigs (Jensen, 2002). Nevertheless, despite the similarity in behavioural expression between play fighting and real fighting, the former does not usually result in injury and losers are willing to initiate new bouts of play fighting (Pellis & Pellis, 2016, 2017). Instead, real fighting, even between immature individuals, may result in injuries (Rushen & Pajor, 1987).

In this study we test the hypothesis that play fighting and real fighting in domestic pigs converge on serving a similar function. To this purpose, we gathered behavioural data on play fighting in three litters of domestic pigs (in the period before weaning) and built sociomatrices and social networks based on both play and real fighting. The social network analysis allows the precise definition of the network structure and the position of each individual within the structure beyond the dyadic interaction level for a given behavioural system (Büttner, Czycholl, Mees, & Krieter, 2020; Foister et al., 2018; Lutz, Ratsimbazafy, & Judge, 2019). We formulated the following predictions.

Prediction 1 - Play opening. Wild boars and domestic pigs (*Sus scrofa*), as well as some phylogenetically closely related species (e.g. warthogs: *Phacochoerus africanus*; Visayan warty pigs; *Sus cebifrons;* Pellis and Pellis, 2016, 2017), can use both contact and non-contact motor patterns to open a play session. Starting a play session by using a contact pattern seems to provide an advantage to the subject as it increases the probability to outcompete the partner (Vanderschuren

et al., 2016). If in domestic pigs play fighting functions as a substitute for real fighting, we expect that piglets are less likely to accept play fighting invitations when the playmate tries to gain an immediate advantage by using body-contact to open the play session.

Prediction 2 – Play maintenance. In domestic pigs, body size is crucial to determine the dominance status and the access to resources, with larger and heavier individuals more frequently winning the agonistic interactions (Andersen, Nævdal, Bakken, & Bøe, 2004; Andersen et al., 2000; D'Eath 2002; D'Eath & Turner, 2009; Jensen, 1988; Norring, Valros, Bergman, Marchant-Forde, & Heinonen, 2019; Turner & Edwards, 2004). The asymmetry in body size between partners can affect the aggressive contest duration because as disparity in weight increases the fight duration decreases (Riechert 1998). If, in piglets, play fighting functions as a substitute for real fighting, we predict that the duration of the playful sessions decreases if the asymmetry in body size between players increases, as it occurs during aggressive encounters (*Prediction 2*).

Prediction 3 - Play ontogeny. In mammalian species with rapid growth, when social play between immature subjects mainly serves long-term more than immediate functions (e.g. social assessment, motor training useful during adulthood), play fighting is usually the main form of interaction until weaning, with aggressive interactions increasing at a later stage (Camerlink, et al., 2018; D'Eath &Turner, 2009; Fagen, 1981). Moreover, the outcomes of play fighting can be used, in some species, to establish dominance relationships, as it occurs with real fighting during adulthood (Blumstein et al., 2013; Weller et al., 2020). The structure of aggressive social networks in piglets is defined by the centrality and level of connections from/to each subject (Büttner et al., 2020). In this view, if piglet play fighting functions as a substitute for real fighting, we expect that: i) play fighting may be rapidly replaced with real fighting during the early phases of development (*Prediction 3a*); ii) at the dyadic level, the outcome of play fighting (i.e. being the winner or the loser) may match the outcome of real fighting (*Prediction 3b*); and iii) at the social network level,

the centrality and influence of a subject with respect to others is similar between the networks of play and real fighting (*Prediction 3c*).

METHODS

Ethic statements

The present research was approved by the Department of Life Sciences and System Biology (DBIOS) of the University of Turin (Italy). The research was purely observational and non-invasive; therefore, the necessity of specific permit is waived. This study is part of the broader project "*So.Pig*" (Department of Life Sciences and Systems Biology, University of Turin). This project focuses on domestic pigs that are not raised under intensive farming, with the aim of better understanding social dynamics and their relationship with welfare, the effect of domestication on behaviour, and possible evolutionary divergences and convergences with other social species, including humans. The subjects under study were reared in an ethical farm (*Parva Domus*, Turin, Italy) and were housed in large enclosures that were parts of natural habitat with a freely accessible indoor and outdoor spaces. The enclosures were also enriched with straw, sheds and troughs. Piglets stayed with their mothers until weaning. Sows and piglets were able to move freely and avoid each other if wanted. The study did not require that sows and piglets be removed from their group either temporarily or on a longer-term basis. Piglets could perform their ordinary behavioural repertoire; no aberrant or stereotypic behaviours were recorded. To ensure welfare control, the farmer weighed piglets at birth and, then, every two weeks until their weaning week. Periodically, vet visited the pigs for vaccination or health problems.

The authors took all the possible precautions to minimize the imposition of fear, distress or lasting harm on pig and to reduce the impact of their presence on animal environment. During the first week after birth the piglets were habituated to the presence of the observers. The authors did not interact with piglets, which were free to perform their ordinary maintenance and social activities. For identification purposes, no semi-permanent or permanent markers were used (e.g. tag, tattoo,

157 freeze marking or branding). The authors marked piglets with animal painting spray (Raidex ©) and
158 marking was renewed every 4-7 days depending on weather conditions. This process was fast and it
159 did not involve physical contact with piglets.
160
161 *The study group*
162 The research was carried out on three domestic pig litters (Parma Black x Parma Black and Parma
163 Black x Large White) hosted at the ethical farm *Parva Domus* (Cavagnolo, Torino - Italy). The
164 study included a total of 24 piglets (11 females, 13 males; Table A1) with different mothers (Linda,
165 Nina, Black Beauty) and same father (Bob). The three sows were kept in individual enclosures with
166 their offspring until weaning, which occurred at around 8 weeks of life. Each enclosure measured
167 around 100 m^2 and was located in an area of natural habitat equipped with straw, troughs for food,
168 water and a shed. The individuals were able to freely move throughout the enclosure and avoid
169 conspecifics if wanted. Sows received food pellets (Ciclo Unico P, SILDAMIN®) each morning
170 between 8:30-10:30 am, whereas the maternal milk represented the major food source for piglets
171 until weaning. No food was specifically given to piglets before weaning although they could
172 opportunistically feed on the pellets given to the sow. Piglets could supplement maternal milk with
173 roots, leaves and fruits found in the natural environment or with pellets left by their mother. The
174 tails and teeth of piglets were kept intact and males were castrated during the first three days of life.
175 The animals followed the natural day/night cycle and did not perform any aberrant or stereotyped
176 behaviour.
177
178 *Data collection and operational definition*
179 The behavioural patterns of piglets were video-recorded from September until December 2018
180 using HD/Full HD Sony HDR-XR200 and Panasonic HC-W3580 cameras, for a total of 130 hours
181 of videos collected for all three litters (Linda's litter = 46.5 hrs, Nina's litter = 40.5 hrs, Black
182 Beauty's litter = 43 hrs). During the first week after birth the observers underwent a training period

to become skilled at animal identification and behavioural pattern distinction. In the same week, piglets were habituated to the presence of the observers. Actual data collection started on the 6th day of piglet life. Behavioural observations took place 6 days per week and ranged from 08:00 am to 05:00 pm. During the video collection the observers stayed at a distance of around 10 meters from piglets and sows. To facilitate the identification of each subject, piglets were marked with animal painting spray (Raidex ©) 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. To ensure welfare control, the farmer weighed the piglets at birth and, then, every two weeks until their weaning week via a mechanical human body weighing scale (Health-o-meter 160LB Professional Floor Scales). Thus, we were able to evaluate the weight difference between subjects, which remained constant from the first until the fourth weighing session (Spearman correlation test between $weigh_{first}$ - $weigh_{fourth}$: N = 24, r = 0.842, P < 0.001). The subjects were divided into weight categories obtained by dividing piglet weights in tertiles and assigned each individual to a specific tertile (1, 2, 3). Two piglets of the same category had a weight difference of 2.5 Kg ± 1.77 (mean ±SD).

By using the all occurrences animal sampling method (Altmann, 1974) we collected 400 play sessions and 386 aggressive interactions between piglets. Moreover, we employed focal animal sampling (Altmann, 1974) to gather data on body contact and grooming events. Prior to starting the video-analysis, M.G. was supervised by G.C. and I.N. in behavioural coding and the video analysis started when the inter-observer reliability scores measured via Cohen's k reached 0.83. 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 videos were analysed frame-by-frame using the program VLC 2.2.1 (*Jump-to-time* extension). For each playful/aggressive event, we recorded: i) the identity of the subjects involved, ii) individual features (gender, age), iii) behavioural patterns performed (Table 1), iv) time of each pattern and v) only for play, the length of the session (seconds).

209 Some of the patterns described in the Table 1 are present in both play and real fighting (e.g. biting,
210 pushing, lifting and head knocking). However, according to previous reports, play fighting is
211 characterised by behavioural elements that are specifically found in the play context and not in the
212 aggressive context. These play markers are: scamper, pivot, head-tossing, and object-shaking
213 (Horback, 2014; Newberry et al., 1988; Rauw, 2013; Ŝpinka, 2017; Table 1). Therefore, we
214 categorized as play fighting all the interactions in which the subjects performed at least one such
215 play patterns. On the contrary, if no play marker was present, the interactions were categorised as
216 real fighting. A play session started when a piglet directed any playful pattern (see Table 1) toward
217 the littermate and finished when both players stopped the interaction, with one of them moving
218 away or with a third subject interrupting the session (Palagi, 2008). Two consecutive sessions were
219 considered as different if the play interruption lasted at least 10 seconds (Cordoni, Nicotra, &
220 Palagi, 2016; Cordoni, Norscia, Bobbio, & Palagi, 2018).
221 A play invitation (PINV) occurred when a piglet approached a companion (the receiver), performed
222 a playful pattern toward it and waited or ran away. If, in turn, the receiver responded with another
223 playful pattern and began the session, the invitation was considered as successful (PINV);
224 conversely, if the receiver ignored the companion and the play session did not occur, the play
225 invitation was considered as unsuccessful (PINV-).
226 For the analysis of PINV, we distinguished the playful patterns into body contact (C) and
227 locomotor-acrobatic (LA, without any body contact). Furthermore, we classified play patterns as
228 offensive (O, unidirectional patterns of attack, directed by one individual toward another), defensive
229 (D, patterns of body protection or contact avoidance) and neutral (N, neither offensive nor defensive
230 patterns) (Table 1).
231 In order to quantify the level of play asymmetry, for each session we calculated the Play
232 Asymmetry Index (PAI; Cordoni et al., 2016, 2018) as follows: the proportion of "wins" for piglet
233 A was subtracted from the proportion of "wins" for piglet B divided by the total number of playful
234 patterns performed by both piglets. The proportion of "wins" for subject A was defined as offensive

patterns by A towards B plus the defensive patterns by B toward A. B's "wins" were calculated in the same way. The PAI ranges from -1 to +1 with zero value indicating a complete symmetry of the session.

$$PAI = \frac{(offensive_{A\rightarrow B} + defensive_{B\rightarrow A}) - (offensive_{B\rightarrow A} + defensive_{A\rightarrow B})}{(offensive_{A\rightarrow B} + defensive_{B\rightarrow A}) + (offensive_{B\rightarrow A} + defensive_{A\rightarrow B}) + neutral_{A+B}}$$

The dyadic hourly frequencies of body contact and grooming events (Table 1) were used to evaluate relationship quality between siblings.

Following de Vries (1993), we built squared matrices including the outcomes of dyadic interactions (number of interactions won or lost by each individual). The matrices were built for both play fighting and real fighting. For each agonistic event we identified a "winner" - the subject that never displayed submissive and/or fear patterns (e.g. fleeing) - and a "loser" - the subject that displayed submissive and/or fear patterns. For each playful event, we defined as "winner" the subject that performed more offensive and less defensive patterns and as "loser" the subject that received more offensive patterns and/or showed more defensive patterns (offensive and defensive patterns are defined in Table 1). Only for the purpose of this analysis, we specifically considered the events in which there was a clear discrimination between winner and loser (de Vries, 1998).

Finally, we defined three periods spanning 14 days of piglet life from the 6th day to the 50th day of life: T_1 (6-20 days; Linda's litter = 16.0 hrs of videos, Nina's litter = 14.0 hrs of videos, Black Beauty's litter = 14.5 hrs of videos), T_2 (21-35 days; Linda's litter = 14.0 hrs of videos, Nina's litter = 12.0 hrs of videos, Black Beauty's litter = 13.5 hrs of videos) and T_3 (36-50 days; Linda's litter = 16.5 hrs of videos, Nina's litter = 14.5 hrs of videos, Black Beauty's litter = 15.0 hrs of videos).

Statistical analyses

Prediction 1

To verify which class of play invitation patterns (i.e. locomotor/acrobatic or body contact patterns) were most likely to be accepted by fellows and followed by play fighting, we employed the non-

parametric Wilcoxon's test corrected for ties for two dependent samples comparison (Siegel & Castellan, 1988). Moreover, for determining which specific type of motor action (see Table 1) was mainly performed for inviting to play, we applied the non-parametric Friedman test for k-dependent sample comparison. We employed the Bonferroni-Dunn *post-hoc* test for pairwise contrasts (Siegel & Castellan, 1988). The use of non-parametric statistics was necessary owing to the non-normal distribution of data (Kolmogorov-Smirnov test: $P > 0.05$).

Prediction 2

In order to evaluate which factors potentially affected the duration of piglet play, we ran a Linear Mixed Model analysis (LMM). We tested as dependent variable the mean duration (in seconds) of dyadic playful sessions (Normal distribution; Kolmogorov-Smirnov test $P = 0.10$). The identity of the players was entered as random factors. As fixed factors we included: sex combination (sex_comb, factorial variable: MM = 1; MF/FM = 2; FF = 3), litter identity (group, factorial variable: Linda's group = 1; Nina's group = 2; Black Beauty's group = 3), relationship quality (bonding, factorial variable: 1 = 0.000 ≤ hourly frequency (body contact + grooming) ≤ 2.841; 2 = 2.841 < hourly frequency (body contact + grooming) ≤ 5.333; 3 = hourly frequency (body contact + grooming) > 5.3332), same body weight_different body weight (SW_DW, factorial variable: 1 = Same Weight_SW; 2 = Different weight_DW) and Play Asymmetry Index (PAI, scale variable).

We tested models for each combination of variables, spanning from the null (the intercept only) to the full model (all the independent variables included). We applied the Akaike's Corrected Information Criterion (AICc) to select the best model that is the model with the lowest value of AICc (Symond & Moussalli, 2011). Then, we calculated the difference (ΔAICc) between the lowest AICc value and the AICc value of each other model. We considered as competing models those showing a ΔAICc ≤ 2. For assessing the strength of each candidate model, we employed ΔAICc to calculate the evidence ratio and the Akaike weight (*w*i), ranging from 0 to 1. The *w*i is the weight of evidence or probability that a given model is the best model, taking into account the data and set of candidate models (Symonds & Moussalli, 2011). The evidence ratio of the model weights is

286 calculated by dividing the w_i of the top-ranked model by the w_j of the other models considered
287 (w_i/w_j).
288 The analyses for both *Prediction 1* and *Prediction 2* were carried out via SPSS 20.0.
289 *Prediction 3a*
290 In order to determine the possible difference in the individual hourly frequency of play fighting and
291 individual mean duration of suckling session between the three periods considered (T_1, T_2, and T_3;
292 see *Data collection and operational definition*), we employed the non-parametric Friedman test and
293 the Bonferroni-Dunn *post-hoc* test (non-normal data distribution; Kolmogorov-Smirnov test via
294 SPSS 20.0, $P < 0.05$; Siegel & Castellan, 1988). Moreover, we checked for possible correlation
295 between play fighting and both real fighting and affiliation by using the parametric Pearson
296 correlation test (normal data distribution; Kolmogorov-Smirnov test via SPSS 20.0, P = ns).
297 To test for the possible correlation between the rates of aggression and play fighting at the dyadic
298 level, we ran a correlation via randomization by using the freeware Resampling Procedures (1.3
299 David C. Howell; 10,000 permutations owing to data pseudo-replication (the same individual is
300 included in different dyads)).
301 *Prediction 3b*
302 We checked for the possible correlation between the outcome of real fighting (winner/loser) and the
303 outcome of play fighting via a row-wise correlation test between squared matrices (software
304 Matman 1.0; 10000 permutations). This test makes no underlying assumptions (the smallest sample
305 size for square matrices that can acquire a probability value < 5% is four) and only compares values
306 within the same row, thus overcoming data partial dependency (recurring observations concerning
307 the same individual). The K_r row-wise matrix correlation coefficient is calculated on the basis of a
308 weighted sum of the correlation between all dyads of corresponding rows in the two sociomatrices
309 and it is defined via Kendall's rank order correlation coefficient (de Vries, 1993).
310 *Prediction 3c*

To check whether the position of a subject in the play fighting network may match with the position of the same subject in the real fighting network, we employed Social Network Analysis (SNA). This method has been recently employed also by Turner and colleagues (2020) to evaluate the positions occupied by pre-weaning piglets in play fighting network and assess the effect of a central play fighting network position on later aggressive physical consequences. By providing different parameters, SNA depicts the group network structure and the position of each subject within this structure (Newman, 2010). We employed the version 9.2 of the open-source and multiplatform software *Gephi* (www.https://gephi.org/, distributed under the dual license CDDL 1.0 and GNU General Public License v3) that is commonly used for network visualization and exploration (Cherven, 2015; Saqr, Fors, & Nouri, 2018). A Social Network (SN) is made of two components: the actors composing the network (nodes) and the relations/interactions between actors (edges). The edges can be distinguished as undirected, when the direction of the interaction from initiator to receiver is not considered or directed when each edge has a clear initiator and receiver. In our study, each piglet represents a node and its playful/aggressive interactions with siblings represent the directed edges. Both the play and aggression SN of each litter is visually represented with a graph called a sociogram that is rendered using the Fruchterman Reingold algorithm (Fruchterman & Reingold, 1991). This is a force-directed layout algorithm that employs physical simulation to represent each node according to connected edges; the resulting visualization depicts nodes in a gravitational way (attraction/repulsion). Then we used the layout algorithm Force Atlas 2 to disperse groups and provide space around the most well connected nodes. The sociogram provides a useful visualization of the overall playful/aggressive interactions in each group, the relations between siblings and their position and role within the network. Different parameters can be calculated for quantifying the prominence of each subject and its value of connection in the SN (Cherven, 2015; Saqr et al., 2018). For each play fighting and real fighting network, we considered the following parameters.

General Network parameters (Cherven, 2015; Saqr et al., 2018)

Modularity - it is a measure of the overall network structure and it evaluates the strength of division of a network into modules (i.e. aggregated groups or clusters based on shared characteristics). A network showing a high level of modularity has many connections between the nodes within each module but fewer connections between the nodes in different modules.

Prestige parameters (Cherven, 2015; Saqr et al., 2018)

Normalized in-degree prestige - it evaluates how likely other nodes directly connect with a specific node (i.e. it refers to interactions where the subject is not the initiator) and it can be considered as an estimate of the size of the ego network: a prestigious node receives many ties from other nodes. The in-degree prestige parameter is normalized on the network size (n-1).

Domain prestige - it evaluates the number or proportion of all nodes that can be directly or indirectly connected with a specific node. It represents a measure of the influence of a specific node as voted by neighbouring nodes.

Centrality parameters (Cherven, 2015; Saqr et al., 2018)

Eigenvector centrality - it is an extension of in-degree prestige because it evaluates the prominence of a node considering its neighbouring nodes: a node is important if it is linked to other important nodes. This parameter assigns relative scores to all nodes in the network based on the rationale that connections to high-scoring nodes contribute more to the score of a specific node than equal connections to low-scoring nodes. In this light, a node connected to prominent nodes will have higher values of Eigenvector centrality.

Bridging centrality - a node lying between modules (i.e. aggregated groups/clusters, see Modularity definition) is defined as a bridging node. The bridging nodes in a graph are determined based on their higher values of bridging centrality compared to those of the other nodes. The bridging centrality of a node is the product of the Betweenness centrality that quantifies the number of times a node acts as a bridge along the shortest path between two other nodes.

We checked for a possible correlation between play and aggressive parameters by using both Spearman (for non-normally distributed data: Kolmogorov-Smirnov test, $p<0.05$)) and Pearson correlation test (for normally distributed data: Kolmogorov-Smirnov test, p=ns) via SPSS 20.0.

RESULTS

Prediction 1 - Play opening

The analysis of the start of play fighting showed that the proportion of successful Play Invitation (PINV) performed with locomotor/acrobatic (LA) patterns was significantly higher compared to the proportion of PINV performed with contact (C) patterns ($PINV_{LA}/PINV_{TOT}$ vs $PINV_{C}/PINV_{TOT}$; Wilcoxon exact test $N = 24$, T = 70.0, ties = 1, $P = 0.038$; Figure 1). In particular, a significant difference was found between the proportion of the different types of LA patterns ($PINV_{LAx}/PINV_{LAtot}$) employed in PINV (Friedman test$_{df=23}$ $\chi^2 = 52.885$, $N = 24$, $P < 0.001$). Specifically, the proportions of play run, pivot/scamper and head tossing were higher compared to those of object play, play kneeling/flopping and play laying down (only significant results are reported, Bonferroni-Dunn post-hoc test: $N = 24$, play laying down vs pivot/scamper Q = 1.891, $P = 0.009$; play laying down vs play run Q = 2.109, $P = 0.002$; play laying down vs head tossing Q = 2.348, $P < 0.001$; object play vs pivot/scamper Q = 1.761, $P = 0.021$; object play vs play run Q = 1.978, $P = 0.005$; object play vs head tossing Q = 2.217, $P = 0.001$; play kneeling/flopping vs pivot/scamper $P = 0.06$; play kneeling/flopping vs play run Q = - 1.804, $P = 0.016$; play kneeling/flopping vs head tossing Q = 2.043, $P = 0.003$).

The proportion of unsuccessful Play Invitation (PINV-) performed with C patterns were significantly higher compared to the proportion of PINV- performed with LA patterns ($PINV\text{-}_{LA}$/ $PINV\text{-}_{TOT}$ vs $PINV\text{-}_{C}$/ $PINV\text{-}_{TOT}$; Wilcoxon exact test $N = 24$, T = 23.0, ties = 2, $P = 0.001$; Figure 1).

Prediction 2 - Play maintenance

386 The LMM analysis on the distribution of the mean duration of play fighting sessions (dependent
387 variable, normal distribution) showed three competing models (Table 2). The first model (AICc =
388 114.99; 32.3% of probability to be the best model) included the SW_DW variable. The second
389 model was the null model (intercept only, AICc = 116.11; 18.4% of probability to be the best
390 model). Finally, the third model comprised the variables PAI and SW_DW (AICc = 116.25) and
391 had a 17.1% of probability of being the best model. The variable SW_DW was statistically
392 significant with same-weight dyads playing longer than different-weight dyads. The full model was
393 the worst (AICc = 124.41). See Table A2 for the w_i and evidence ratio values of the models tested.
394
395 *Prediction 3 - Play ontogeny*
396 The individual hourly frequencies of play fighting significantly differed across the three age-periods
397 considered (Friedman test$_{df=2}$ χ^2 = 21.906, N = 23, P < 0.001). In particular, the play rates decreased
398 from T_1 to T_3 (Bonferroni-Dunn post-hoc test: T_1 vs T_2 Q = 0.152, P = 1.000; T_1 vs T_3 Q = 1.217, P
399 < 0.001; T_2 vs T_3 Q = 1.065, P = 0.001).
400 In order to quantify the time spent by piglets in suckling sessions, we calculated the individual
401 mean duration (in minutes) of sessions in each of the periods considered. The suckling duration
402 significantly changed across the three periods (Friedman test$_{df=2}$ χ^2 = 37.130, N = 23 P < 0.0001).
403 Specifically, the duration of suckling sessions significantly decreased from T_1 to T_2/T_3 (Bonferroni-
404 Dunn post-hoc test: T_1 vs T_2 Q = 1.261, P < 0.0001; T_1 vs T_3 Q = 1.739, P < 0.0001; T_2 vs T_3 Q =
405 0.478, P = 0.314; Figure 2).
406 The comparison between play fighting and real fighting rates at the dyadic level showed a
407 significant difference across the three periods, with play fighting prevailing in T_1 and real fighting
408 in T_3 (Wilcoxon exact test: T_1 - play fighting > real fighting, N = 39, T = 12.5, ties = 21, P = 0.001;
409 T_2 - play fighting ≈ real fighting, N = 39, T = 30, ties = 26, P = 0.297; T_3 - real fighting > play
410 fighting, N = 39, T = 0.0, ties = 23, P < 0.001; Figure 3). Moreover, in all the periods considered,
411 the rates of real fighting positively correlated with the rates of play fighting at the dyadic level

(Correlation via Randomization: T_1 N = 39, r = 0.770, P < 0.001; T_2 N = 39, r = 0.522, P = 0.001; T_3 N = 39, r = 0.335, P = 0.046). The analysis at the individual level carried out on the entire observation period, showed that the level of play fighting positively correlated with the level of real fighting but not with the level of affiliation (Pearson correlation test - Bonferroni correction α = 0.025: play fighting - real fighting N = 24, r = 0.695, P < 0.001; play fighting -affiliation N = 24, r = - 0.124, P = 0.564).

We also found a significant correlation between real-fighting and the play fighting sociomatrices for Linda' and Nina's litter (row-wise permutation test: Linda's litter: N = 10, K_r = 61, Tau_{rw}= 0.219, P = 0.014; Nina's litter: N = 8, K_r = 42, Tau_{rw} = 0.393, P = 0.003) and a trend was observed for Black-Beauty's litter (row-wise permutation test K_r = 12, Tau_{rw} = 0.261, N = 6, P = 0.098). Hence, in the majority of cases, the dyadic outcome of agonistic encounters (winner/loser) was correlated with the dyadic outcome of play fighting.

Finally, the Social Network Analysis (Figure 4) revealed that play fighting and aggression social networks positively correlated with respect to centrality and bridging measures (Bonferroni correction α = 0.05/4= 0.0125; Spearman correlation test: Domain prestige N = 24, r_S = 1.0, P = 0.01; Pearson correlation test: Normalized in-degree prestige N = 24, r = 0.693, P < 0.001; Eigenvector centrality N = 24, r = 0.611, p = 0.001; Bridging centrality N = 24, r = 0.521, P = 0.008). Summing up, the piglets that occupied a central or bridging position in the play fighting social network had a similar position in the real fighting social network.

DISCUSSION

In the current study we demonstrated that, during the pre-weaning period, piglets: i) engaged preferentially in play fighting when locomotor-acrobatic play invitation were involved (*Prediction 1* confirmed), ii) performed playful interactions for longer with same-weight partners (*Prediction 2* confirmed), iii) rapidly replaced play- with real-fighting sessions (*Prediction 3a* confirmed), iv) consistently won or lost play- and real fights (*Prediction 3b* confirmed), and v) occupied the same

central or bridging positions in the play- and real-fighting social networks (*Prediction 3c* confirmed). These results are discussed in detail below.

The first step to engage in a playful interaction is represented by a positive response to a play invitation (PINV) by the partner. In line with our expectation (*Prediction 1*), piglets reached the goal of successfully starting a play fight by employing locomotor-acrobatic rather than body-contact patterns to invite the potential playmate (Figure 1). Moreover, unsuccessful PINVs were significantly more frequent if play fighting attempts were introduced by body-contact actions (Figure 1). The preferential use of locomotor/acrobatic patterns can be a strategic way to protect both potential players, which is expected if play fighting is a substitute for real fighting. Piglets may use exaggerated non-contact motor patterns (i.e. play run, pivot/scamper and head tossing) – typical of play (Horbach, 2014; Newberry et al., 1988; Rauw, 2013) – to avoid the risk that the subsequent play behaviour is misinterpreted by the partner (Bekoff, 1995, 2001; Palagi et al., 2016a,b). Moreover, such patterns can ensure a "security distance" between interacting individuals and facilitate the escape opportunity in case the receiver responds aggressively. Similarly, in canids, the play bow can be employed not only as a play signal but also as a strategic posture that the bower may use to flee more easily from the partner (Pellis & Pellis, 1996).

After the beginning of play fighting, it is important to maintain the session for as long as possible trying to maximise its benefits but at the same time limit the risk of being harmed (Pellis & Pellis, 1996, 2009, 2017). In piglets, play fighting sessions were longer when they occurred between players with similar body weight (*Prediction 2*), which highlights the competitive nature of the interaction. As a matter of fact, in pigs heavier individuals (both adult males and sows) win the majority of conflicts and acquire higher social ranks (Andersen et al., 2004; D'Eath 2002; Norring et al., 2019; Turner & Edwards, 2004). Weller and colleagues (2020) found that the piglets that experienced high levels of play fighting then based their aggressive escalation decision mainly upon their own fighting abilities (Resource Holding Potential; Parker, 1974) with the heavier and larger subjects winning the contest. Hence, by play fighting preferentially with similar sized mates, piglets

464 can reduce the probability of losing the contest, thus prolonging the session. In this way, piglets
465 may be able to set the strategy to manage the interaction with the same partners also during
466 aggressive confrontations.
467 Regarding play ontogeny, our results highlight that in the piglets under study play fighting was
468 rapidly replaced with real fighting during the early phases of development (*Prediction 3a*).
469 Intriguingly, the decrease of play before weaning came after the decrease of the duration of suckling
470 sessions. A hypothesis that may deserve further investigation is that the decrease of social play in
471 piglets might be related to a decrease in oxytocin, a neuropeptide that is consumed via maternal
472 milk in immature mammals (Mishra, Ali, & Das, 2014; Prakash, Paul, Kliem, Kulozik, & Meyer,
473 2009) and is generally involved in proximate mechanisms of social affiliation (de Dreu, 2012; Insel,
474 2010; Shamay-Tsoory et al., 2013), and social play (Vanderschuren et al., 2016). Consistently,
475 Martin and Bateson (1985) found that in the domestic cat (*Felis catus*) certain aspects of play
476 (although not necessarily social play) were influenced in kittens by reduced lactation. Romero and
477 colleagues (2015) showed that dogs sprayed with oxytocin played more and for longer periods
478 compared to the control condition. Despite some debates and mixed findings on the effect of
479 oxytocin on the behaviour of domestic pigs (Camerlink, Reimert, & Bolhuis, 2016; Rault et al.,
480 2013; Rault, Dunshea, & Pluske 2015), with respect to play fighting we cannot exclude that
481 agonistic interactions might be maintained in their "playful form" under the effect of oxytocin,
482 which in pigs is highest at early stages of lactation (López-Arjona et al., 2020; Rojkittikhun et al.,
483 1993). This aspect may represent an interesting research line to explore in the future.
484 Moreover, the fact that real fighting increased as play fighting decreased and that play fighting was
485 correlated with the aggression levels but not with the rates of other affinitive patterns further
486 suggest that play fighting in pigs has a competitive more than a cooperative function. We have also
487 to highlight that the males of our study group were castrated. Although these males can still fight
488 for dominance (Foister et al., 2018), we cannot exclude that castration may have had a negative

impact on the development of aggression, leading to an underestimate of the competitive aspects of play. In intact pigs, the aggressive nature of play fighting could be even more pronounced.

The sociomatrix correlation revealed that the outcomes of play fighting interactions (winner/loser) matched with the outcomes of aggressive encounters (winner/loser) (*Prediction 3b*). Moreover, by examining the social impact of piglets on their groups via the Social Network Analysis (SNA), we highlighted a positive correlation between the centrality parameters of play and aggression (i.e. Normalized in-degree prestige, Domain prestige, Eigenvector centrality, Bridging centrality*;* *Prediction 3c*). This result indicates that the position of central or influence occupied by piglets in the play fighting network is maintained in the real fighting network. Overall the above findings highlight that a sort of *continuum* from play to aggression may exist and further supports the hypothesis that play fighting is a substitute for real fighting. The direct connection between play fighting and aggression is also supported by the recent study of Weller and colleagues (2020). These authors showed that the piglet dyads experiencing high levels of play fighting during the pre-weaning period were better able to gather and act upon information regarding their partners' skills than dyads experiencing low levels of play fighting. In this respect, subjects can reciprocally assess their own abilities in a less costly and safer manner and improve their abilities in making tactical decision in aggressive contests (Parker, 1974; Weller et al., 2020). The findings of the present study confirm our initial hypothesis that play fighting can be a substitute for real fighting. As a matter of fact, piglets engaged in play fighting when their chance to win was highest (similar weight players) and the danger of being harmed lowest (low risk invitation patterns). Importantly, the outcomes of play fighting predict the outcomes of real fighting, with the social network of the two forms of competition (serious and non-serious) largely matching. However, these same findings point towards the more extreme hypothesis that play fighting and real fighting might be mechanistically the same. In this respect, play fighting might be a different qualitative form of real fighting with elements typically regarded as play markers. Even though at this stage of knowledge, the most parsimonious interpretation is considering play fighting as actual play replacing aggression, our

results could also suggest that play fighting might be a form of real fighting performed in a less dangerous way.

In conclusion, what remains unresolved by our research is whether the skills acquired from play fighting involve improved combat ability and/or some form of improved socio-cognitive skills. For example, the greater capacity to win aggression and obtain a dominant position may arise from greater capacity in assessing one's own ability relative to that of the opponent (Parker, 1974; Weller et al., 2020). In other words, the improved ability to assess and maintain dominance relationships may arise from improved socio-cognitive skills rather than improved combat skills. Although the mechanisms underlying the competitive nature of play fighting remain to be determined, our study provides convincing evidence that play fighting in piglets may predict the ability to attain dominance and can be used as a substitute for real fighting.

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764 **Table 1** - Pig playful, aggressive and affinitive behavioural patterns recorded in the current study

765 (integrated or modified from Newberry et al., 1988; D'Eath, 2002; Jensen, 2002; Bolhuis et al., 2005; Rauw, 2013;

766 Ŝpinka, 2017; Weller et al., 2019)

767

PLAYFUL, AGGRESSIVE/SUBMISSIVE AND AFFINITIVE PATTERNS	
Behavioural pattern	**Description**
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Flopping N_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 which could cause the change of position
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Head-tossing N_LA	A piglet gently head shakes from one side to another
Hopping N_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
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Nudge N_C	A piglet uses its snout to gently touch another piglet's body (excluding naso-naso contact). It is more intensive than touching, but also more gentle than pushing
Object play N_LA	A piglet manipulates an item or securely holds it in its mouth, energetically shaking it or carrying it around the pen
Pivot N_LA	A piglet twirls its body on the horizontal plane by a minimum of 90°. Pivot is usually associated with jumping on the spot
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Play fight O_C	Two piglets mutually push in a head to head orientation. It can include attempt play bite, play bite, play push etc.
Play kneeling D_LA	A piglet goes down on its knees while playing
Play lifting O_C	A piglet attempts to displace a partner by lifting or levering it with snout or head
Play lying down D_LA	A piglet places itself in a horizontal position during play
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Play run O_LA	A piglet runs and hops in forward motions within the pen environment. Run can be performed both in solitary and social manner
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	each other usually associated with excitability
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Aggressive bite	A piglet opens its mouth and closes its teeth tight on a small piece of the opponent's flesh (except tail)
Aggressive fight	Two piglets mutually push one another in a head to head orientation. The pattern can include biking, kicking, chasing, pushing, head knocking, ect.
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Aggressive kick	A piglet kicks with one or both hind limbs the opponent, striking it
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Aggressive push	A piglet presses its head, neck, shoulder or body against the opponent in an aggressive context
Attempt aggressive bite	A piglet opens its mouth, directs or turns its head toward the body of the opponent and closes its mouth without contact
Avoidance	A piglet moves away with a depressed tail when the opponent approaches
Chase	A piglet pursues the opponent (for more than a three body-lengths distance)
Displacement	A piglet causes the opponent to move and takes its place at a resource spot
Head tilting	A piglet moves the head to the side when the opponent passes or gets closer
Tail biting	A piglet bites the tail of the opponent
Threat	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
Body contact	Two piglets touch one another with their bodies (but not resting)
Mutual grooming	Two piglets use their teeth to carry out reciprocal hair cleaning
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Social rubbing	A piglet rubs over the body of another
Touch	A piglet touches another with a foot or other body parts

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Table 2 - The results of the best competing models obtaining for the LMM analyses

DEPENDENT VARIABLE: mean duration of play sessions					
Model$_1$ = SW_DW (AICc = 114.986); w_i = 0.323					
Fixed variable	***Coefficient***	***t***	**SE**	***P***	**95%*CI***
SW	0.238	0.108	2.209	**0.03**	0.023/0.453
DW			0[a]		
Intercept	1.932	0.108	17.910	**<0.001**	1.717/2.147
Random factors	***Estime***	**SE**	***Z***	***P***	**95%*CI***
Player $_1$	0.128	0.054	2.342	0.019	0.055/0.295
Player $_2$	0.036	0.028	1.283	0.200	0.008/0.167
Model$_2$ = null model (AICc = 116.111); w_i = 0.184; evidence ratio = 1.755					
Fixed variable					
Intercept	2.020	20.123	0.100	**< 0.001**	1.819/2.220
Random factors					
Player $_1$	0.129	0.057	2.258	0.024	0.054/0.306
Player $_2$	0.037	0.030	1.218	0.223	0.007/0.183
Model$_3$ = *PAI, SW_DW* (AICc = 116.252); w_i = 0.171; evidence ratio = 1.889					
Fixed variables					
PAI	-0.188	0.167	-1.127	0.264	-0.522/0.145
SW	0.253	0.108	2.353	**0.021**	0.039/0.468
DW			0[a]		
Intercept	1.935	0.111	17.402	**< 0.001**	1.713/2.157
Random factors					
Player $_1$	0.143	0.060	2.385	0.017	0.063/0.326
Player $_2$	0.039	0.029	1.379	0.168	0.010/0.163

Table A1 - Composition of the three study litters of domestic pig hosted at the ethical farm *Parva Domus* (Turin, Italy)

PIGLET	SEX	DATE OF BIRTH	MOTHER
MGL	M	16/09/2018	LINDA
LBL	M	16/09/2018	
LVL	M	16/09/2018	
PBL	M	16/09/2018	
PVL	M	16/09/2018	
MAL	F	16/09/2018	
FCL	F	16/09/2018	
XVL	F	16/09/2018	
COL	F	16/09/2018	
OCL	F	16/09/2018	
CAN	M	05/11/2018	NINA
PRN	M	05/11/2018	
MMN	M	05/11/2018	
LVN	M	05/11/2018	
FMN	F	05/11/2018	
LBN	F	05/11/2018	
PBN	F	05/11/2018	
PVN	F	05/11/2018	
LRBB	M	03/10/2018	BLACK BEAUTY
CVBB	M	03/10/2018	
CRBB	M	03/10/2018	
CBBB	M	03/10/2018	
ARBB	F	03/10/2018	
XBB	F	03/10/2018	

799 **Table A2** - The values of AICc, w_i and evident ratio of each model tested in the LMM analysis

800 (duration of the play session = dependent variable, normally distributed)

801

Models	AICc	w_i	evidence ratio
SW_DW	114.986	0.323	*
intercept (null model)	116.111	0.184	1.756
PAI, SW_DW	116.252	0.171	1.884
sex_comb, SW_DW	117.570	0.0889	3.641
PAI	117.820	0.078	4.126
group	118.213	0.064	5.022
sex_comb	119.231	0.039	8.355
bonding	119.553	0.033	9.814
group, bonding	121.749	0.011	29.425
sex_comb, SW_DW, group, bonding	123.416	0.005	67.717
PAI, sex_comb, SW_DW, group, bonding (full model)	124.408	0.003	112.200

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804 **Figure captions**

805 **Figure 1 - *Play opening***

806 Box plot showing the rate of successful (PINV) and unsuccessful (PINV-) Play Invitation in
807 relation to the type of pattern used by a piglet for inviting a littermate to play. C = body contact
808 patterns; L/A = locomotor/acrobatic patterns (no body contact). Solid horizontal lines indicate
809 medians, length of the boxes corresponds to inter-quartile range and thin horizontal lines indicate
810 range of observed values.

811 **Figure 2 - *Duration of suckling sessions***

812 Box plot showing the individual mean duration (in seconds) of suckling sessions in each of the
813 three periods considered (T_1, T_2, T_3) determined on the basis of the days of piglet life. T_1 = 6-20
814 days, T_2 = 21-35 days, T_3 = 36-50 days.

815 **Figure 3 - *Play ontogeny***

816 Box plot showing the hourly frequencies of play/aggressive interactions in relation to the three
817 periods (T_1, T_2, T_3) determined on the basis of the days of piglet life. AGG = aggressive
818 interactions; PL = play interactions; T_1 = 6-20 days, T_2 = 21-35 days, T_3 = 36-50 days.

819 **Figure 4 - *Play and Aggression Sociograms***

820 The play and aggression sociograms of each study litter (Linda, Nina and Black Beauty's litters).
821 Nodes (i.e. piglets) are represented as circles and edges (i.e. play/aggressive interactions) as arrows.
822 Each circle size corresponds to the degree centrality (i.e. quantity of play/aggressive interactions).
823 The two different colours of the nodes (i.e. gray and white) represent the subgroups within each
824 litter determined by the *Modularity* parameter. No subgroups were obtained for the sociogram of
825 aggression in the Black Beauty's litter.

ACKNOWLEDGEMENTS

The authors wish to thank Davide Lovera and Cristina Desderi, managers and owners of the ethical farm "*Parva Domus*" (Cavagnolo, Italy) for their curiosity, support, and availability over the course of the entire study. The authors wish also to thank the entire staff of the farm. Furthermore, the authors would like to thank Edoardo Collarini, Elisabetta Coco and Marta Comin for their support during fieldwork, the two anonymous reviewers and Costanza S. who helped improve the content of the manuscript. This study is part of the broader project "*So.Pig*" (Department of Life Sciences and Systems Biology, University of Turin). This project focuses on domestic pigs that are not raised under intensive farming, with the aim of better understanding social dynamics and their relationship with welfare, the effect of domestication on behaviour, and possible evolutionary divergences and convergences with other social species, including humans. The present study was partly funded by the University of Turin, Department of Life Sciences and Systems Biology, via research funds (code NORI_RILO_18_01).

Figure_1

Click here to access/download;Figure;R3_figure_1.tif

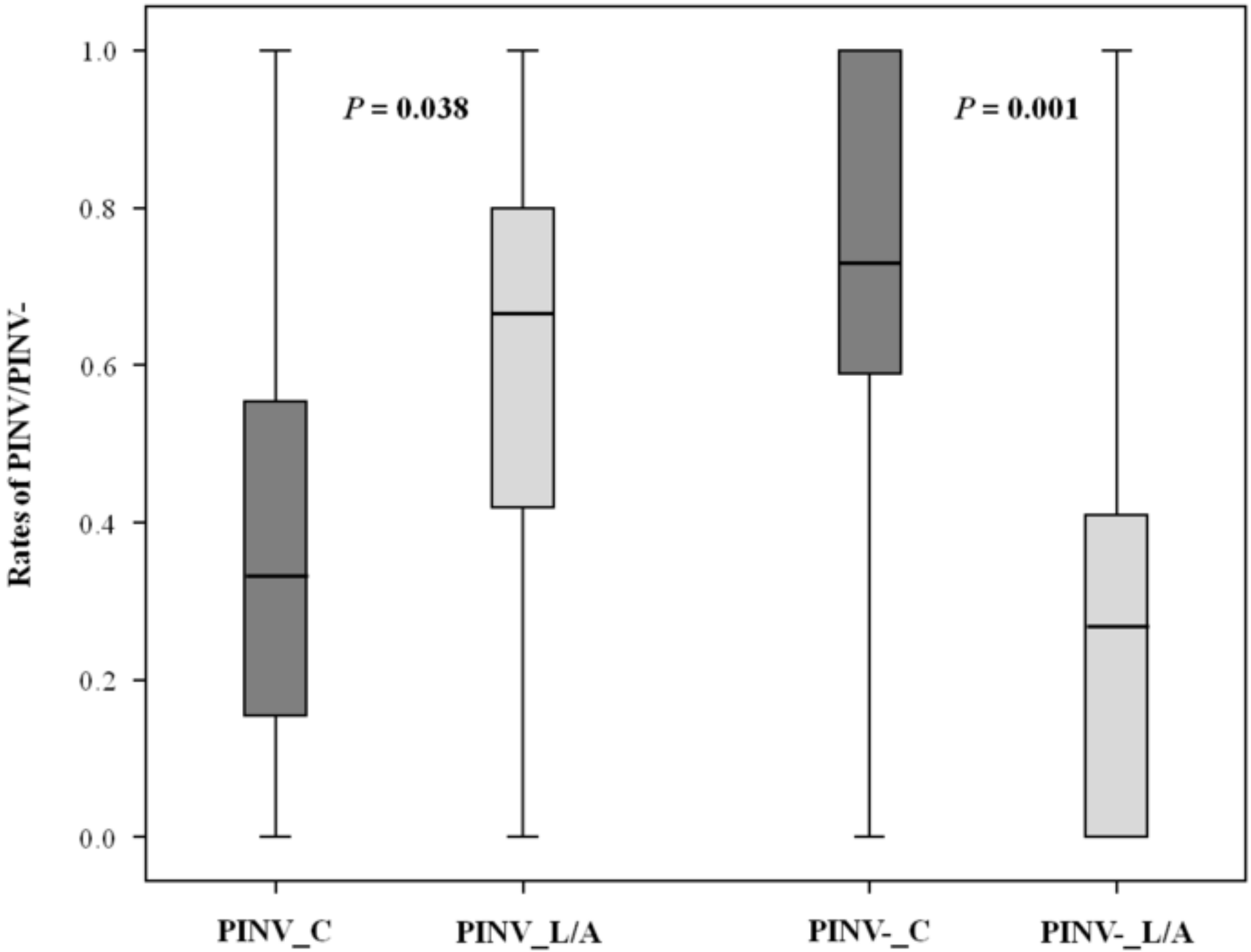

Figure_2

Click here to access/download;Figure;R3_figure_2.tif

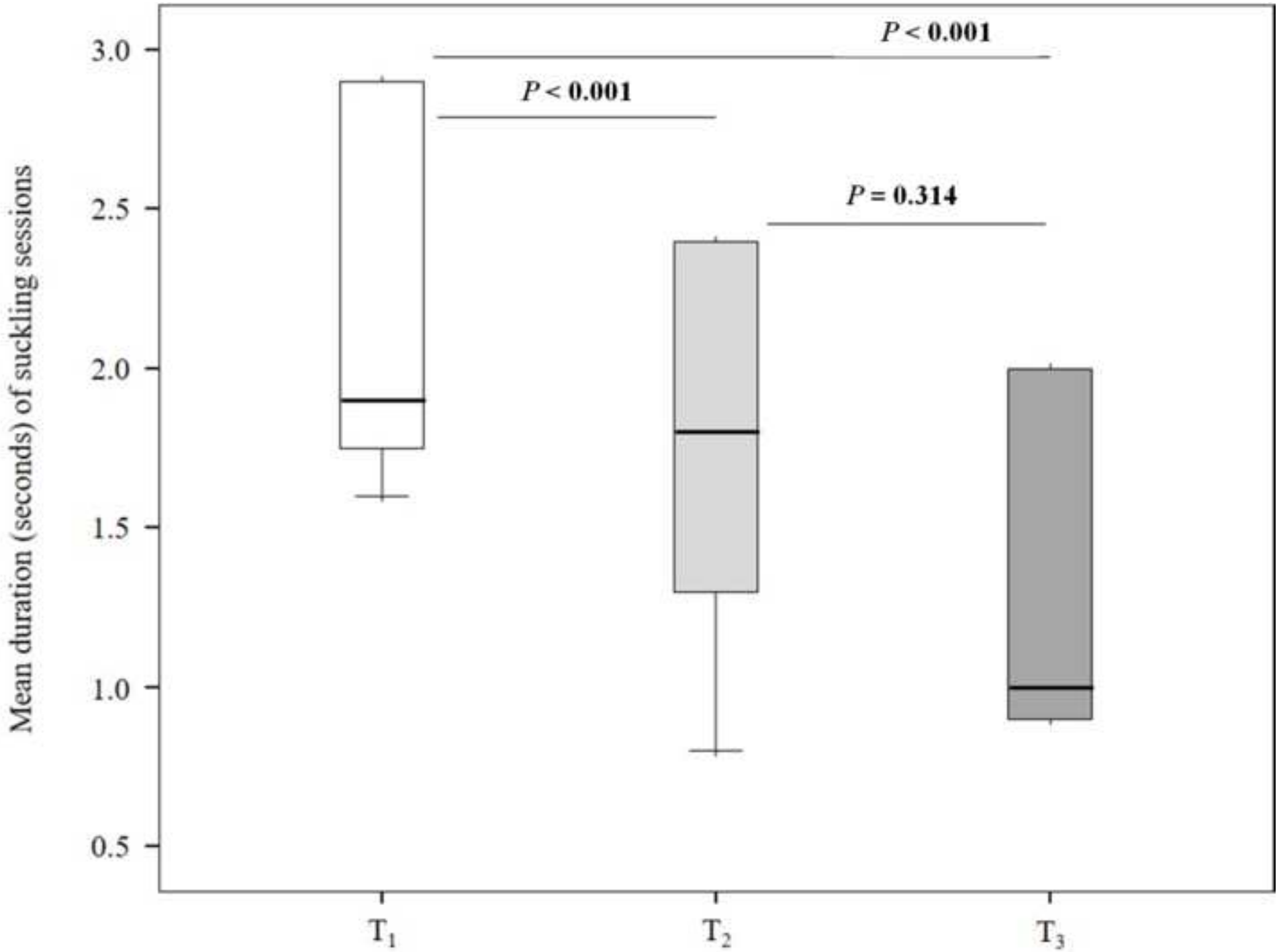

Figure_3

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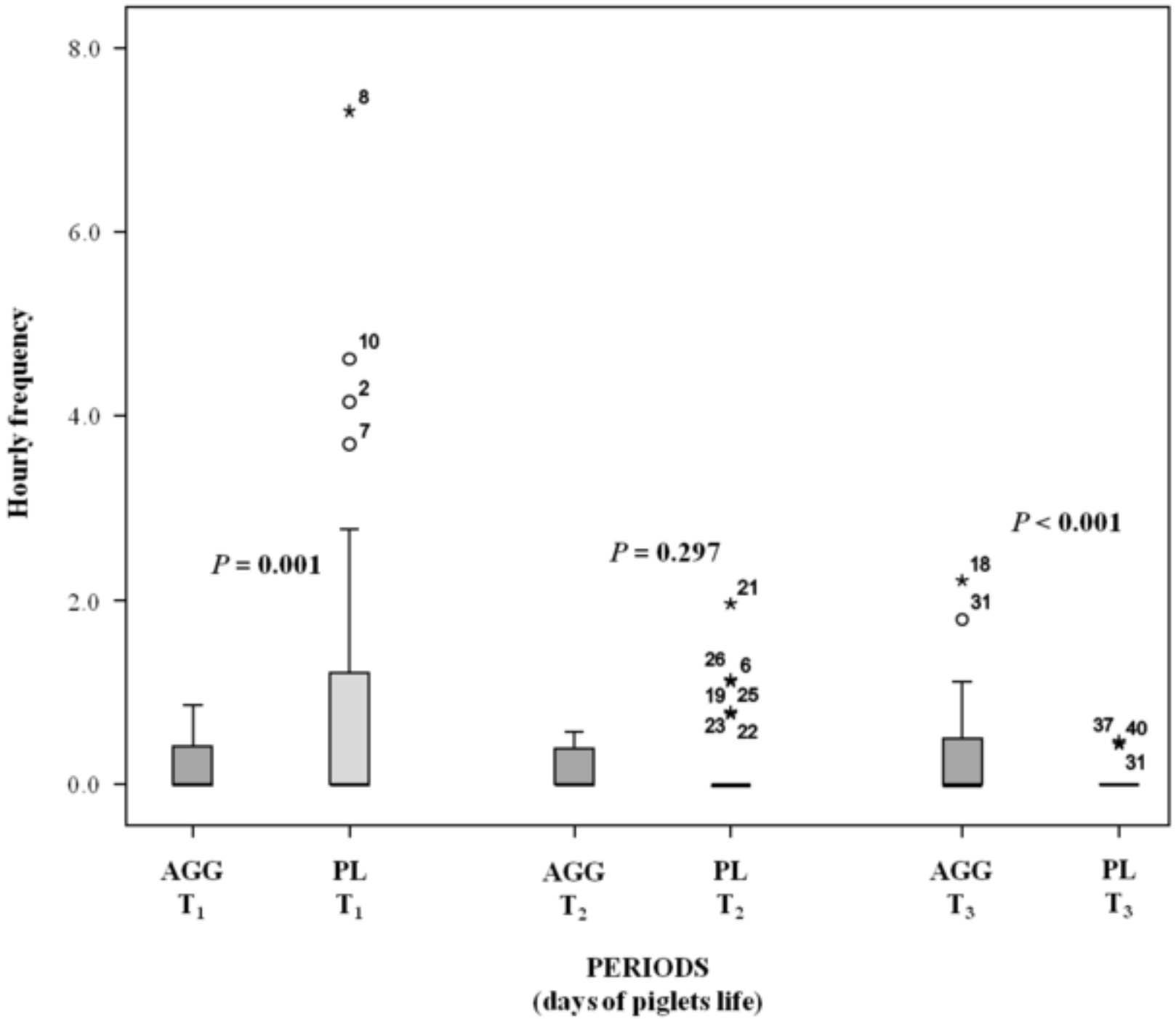
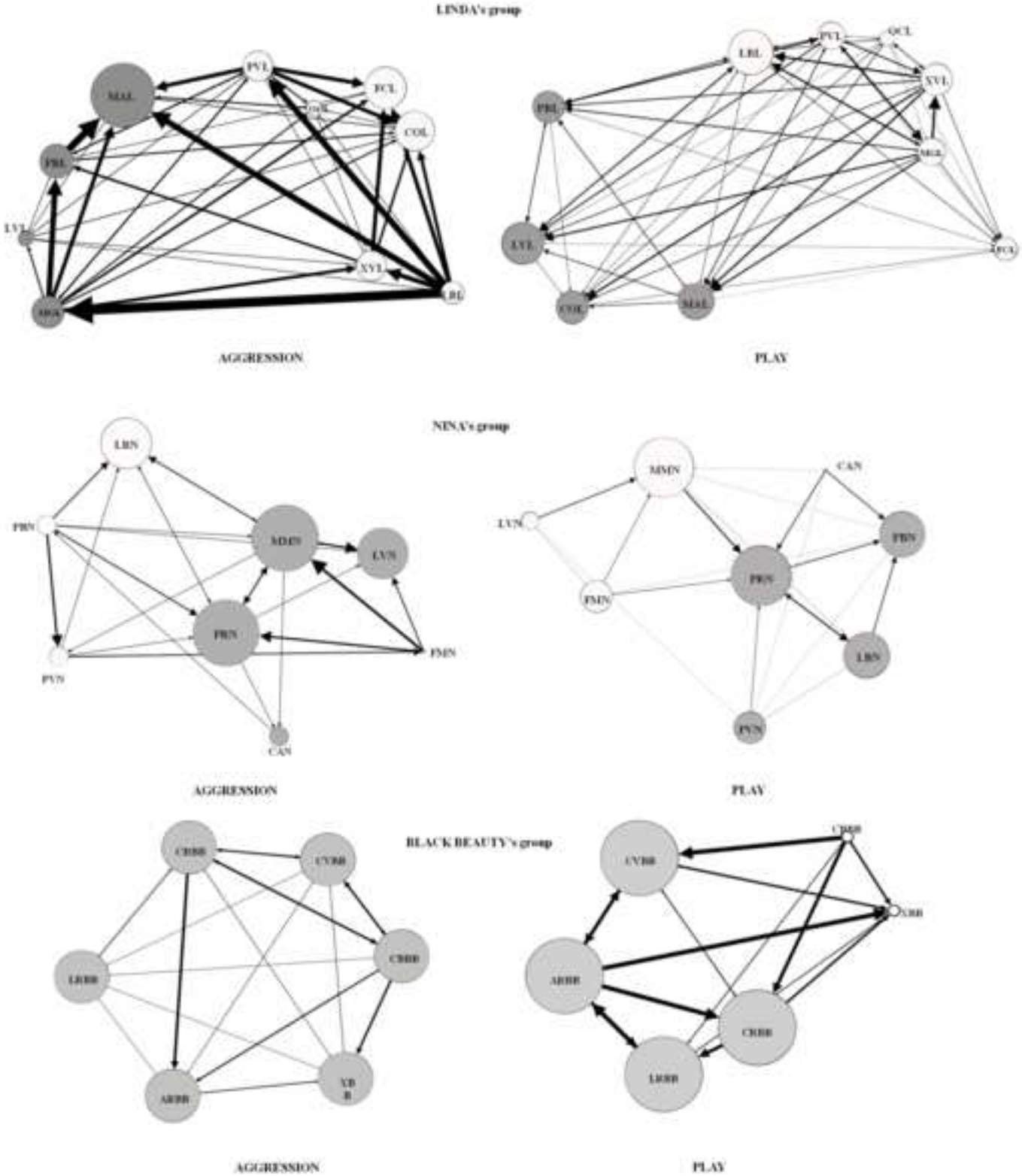


Figure_4

Click here to access/download;Figure;R3_figure_4.tif



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Legend: O = Offensive play pattern; D = Defensive play pattern; N = Neutral play pattern; LA = Locomotor-Acrobatic play pattern; C = Contact play pattern

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