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Socio-emotional communicative modules in parental and alloparental interactions in human and non-human primates

Marta Caselli

Tutor: **Ivan Norscia**

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“Are we smart enough to know how
smart animals are?”
Frans de Waal

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GENERAL INTRODUCTION AND THESIS PLAN

Socio-emotional communication and emotional contagion

Socio-emotional communication can be defined as the entirety of communicative signals that have been selected to elicit positive or negative feedbacks from social partners (Tretter and Diefenbach, 2020). Several socio-emotional and cognitive abilities are needed to efficiently communicate with other, e.g., attention, prosociality, empathic propensity, cooperation, and imitation (Rautakoski et al., 2021). Such abilities are developed during infancy within different social contexts, particularly during mother-infant exchanges and playful interactions with peer conspecifics (Pellegrini et al., 2007; Hrdy, 2009).

In both human and non-human primate societies, socio-emotional communication plays an essential role (Parr et al., 2005; Scholl, 2013). One way to investigate such form of communication is via the study of emotional contagion.

Among several others, three different definitions - proposed by Yamamoto (2017), Decety and colleagues (2016), and Preston and de Waal (2002) - try to explain the mechanisms underpinning emotional contagion. Yamamoto (2017) has proposed a model of empathy that is based on three independent factors: i) matching with others (e.g., motor synchrony, mimicry, emotional contagion), ii) understanding of others (e.g., perspective taking, Schadenfreude) and iii) prosociality (e.g., food sharing). According to this model, these three different factors partially overlap, thus producing different empathy-related phenomena (Yamamoto, 2017; Figure1).

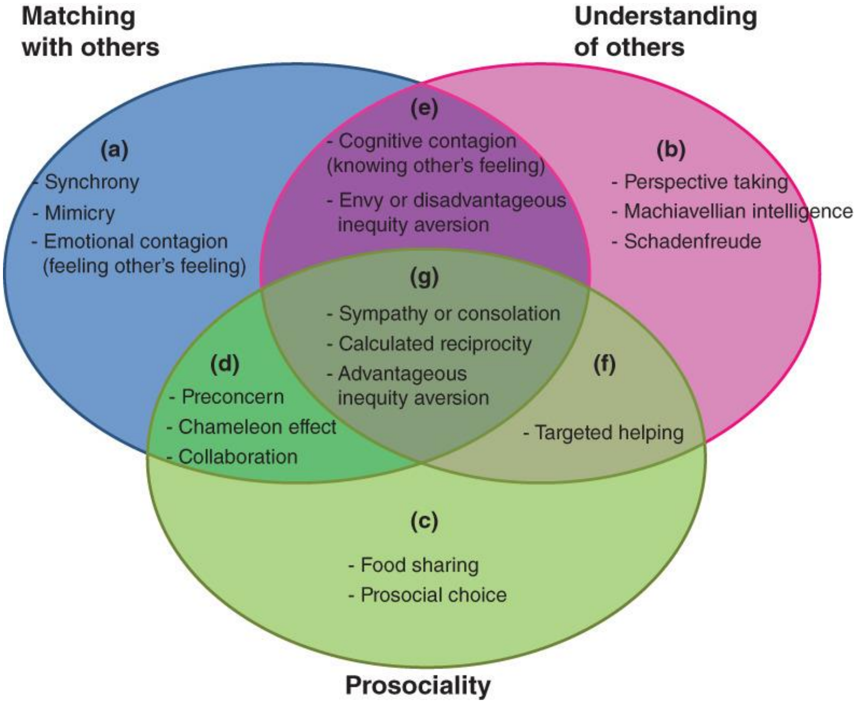

Figure 1 Empathy model as proposed by Yamamoto, 2017

The second definition of empathy - proposed by Decety and colleagues (2016) - suggests that empathy is an induction process that derives from an innate ability to perceive and be sensitive to the emotional states of others. In this view, the capacity to understand others' emotions results from a biological capacity (Figure 2).

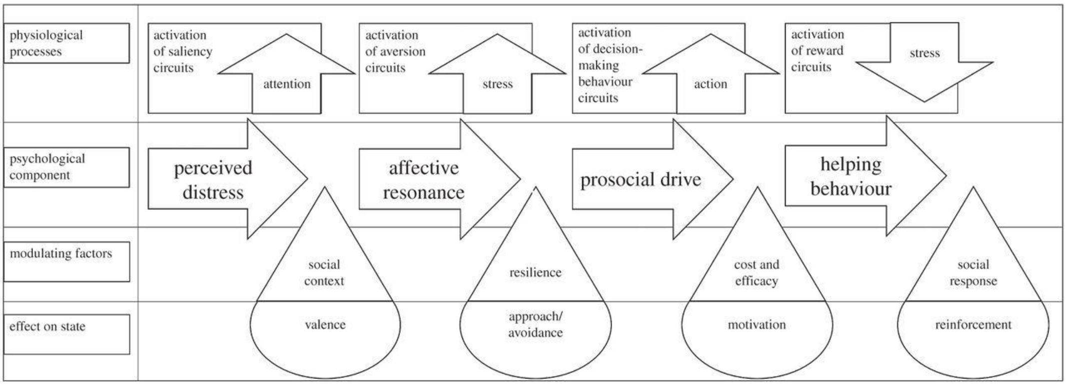

Figure 2 Empathy as a driver of prosociality, as proposed by Decety et al., 2017

The last model is the Russian-Doll model (Figure 3) - proposed by Preston and de Waal (2002) - which is based on the Perception-Action Model (PAM) and on the Mirror Neuron System (MNS). This multilayered model proposes a translation from motor resonance into emotional resonance. The PAM considers the individual experience (de Waal and Preston 2017) while MNS is more focused on the target of an emotionally-charged motor pattern (Rizzolatti and Caruana 2017). The PAM and MNS hypothesize that a subject unconsciously and spontaneously replicates a certain emotionally-charged facial expression (Action) after perceiving the same facial expression of another subject (Perception). Such motor matching creates an “emotional bridge”

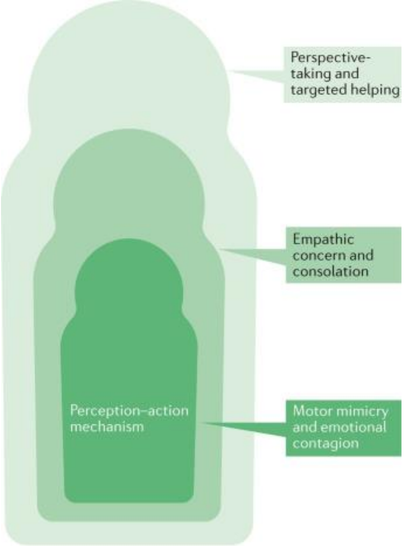

Figure 3 Empathy model as proposed by Preston and de Waal, 2002

between the actor of the facial expression and the responder that, through the activation of a shared neural representation, feels the same emotion that provoked the facial expression in the actor.

All models have in common the fact that a bottom-up approach (sensu de Waal and Ferrari, 2010) to the study of socio-emotional communication can be adopted, by investigating how such communication is expressed in non-human primates and humans. In this thesis, I adopted this approach.

Socio-emotional communication is extremely important in establishing and reinforcing mother-infant bonding. Mother-infant attachment in humans starts long before pregnancy thanks to the physiological and hormonal changes liked to gestation (Napso et al., 2018; Barba-Müller et al., 2019; Tichelman et al., 2019). After birth, in both human and non-human primates, a mother is emotionally affected by and reacts to the emotional expression of her infant and *vice versa* and this nurtures the relationship through a positive feedback that benefits both individuals (Preston and de Waal, 2002). As concerns the benefits obtained by the infant, the emotional and physical contact with the mother has an impact on its future socio-emotional competence (Brazelton et al., 1974; Stern 1974, 1977; Deboer and Boxer, 1979; Levine, 1990; Gable and Isabella, 1992). By becoming able to recognize and understand others' emotions, the infant develops and enhances the skills needed to successfully interact with other conspecifics in several social contexts (e.g., to form alliances, play, reproduce, avoid conflicts etc…) (Preston and de Waal, 2002). On the other hand, the capacity of the mother to detect and recognize the emotional states of her infant, ensures that the mother responds adequately to the needs of her infant (Preston and de Waal, 2002). This capacity is developed by females before becoming reproductive (Pryce, 1992). During puberty, females undergo hormonal changes in preparation for the possibility of a pregnancy. Such changes lead adolescent females to become interested in infants of other females, in order to gain experience with them (Pryce, 1992). This interest continues during the entire life of females, even after becoming mothers themselves (Maestripieri, 2010). The interest females show towards infants, makes mothers more attractive than non-mothers, enhancing female-female interactions (Hrdy, 1976; Maestripieri, 1994).

Thesis plan

To better understand the evolution of human socio-emotional communication, I investigated the phenomenon of emotional contagion via Rapid Facial Mimicry (RFM) and Yawn Contagion. I explored RFM and Yawn Contagion in three different contexts (mother-infant relationship, female-female relationship and infant-infant relationship) in three different primate species: geladas - *Theropithecus gelada* - (which separated from the human line around 20 million years ago, Pozzi et al., 2014), bonobos - *Pan paniscus* - (which separated from the human line around 6-7 million years ago and with chimpanzees share with humans the last common ancestor; Fleagle, 2013) and humans - *Homo sapiens*.

The choice of studying three primate species is in accordance with the principle of Darwinian evolutionary continuity that is also resumed in later theories (e.g., the empathy models described above) as it follows the principle according to which in biology "*the old always remains present in the new*" (de Waal, 2007, pag 49). Therefore, exploring socio-emotional communication in three primate species showing different degrees of phylogenetic closeness, allowed me to contribute to a better understanding of the evolutionary bases of this form of communication. Here below I briefly go through the content of the chapters that compose this thesis.

Chapter 1 - I present a wild population of geladas that had never been studied before. Indeed, a survey was needed to get to know the study population and to individually recognize a sufficient number of study subjects. Moreover, as this gelada population frequented an area with human settlements, I assessed how crop and pasture areas potentially altered health of individuals (including lactating females) and their social (affinitive and aggressive) behavior to be able to focus subsequent research on the population that was less impacted by humans.

Chapter 2 – Here I investigated the communicative patterns and the affiliative exchanges between gelada females, by focusing on the possible role of infants in modifying and modulating females' social interactions. More specifically, I investigated for the first time in geladas whether the presence and occurrence of infant handling modified the amount of grooming females exchanged. In geladas, affiliative behaviors are extremely important, especially between females which represent the core of the social group (Dunbar and Dunbar, 1975). Females' cohesion is maintained via positive interactions, such as agonistic support (Pallante et al., 2016), embracing (Pallante et al., 2019), and a large amount of grooming (Dunbar and Dunbar, 1975; Dunbar, 1983; 2014). Moreover, the tolerant nature of geladas (Dunbar and Dunbar, 1975) makes geladas a good model species to study the presence and possible effects of infant handling on female interactions. Furthermore, because infant handling could act as a 'social bridge' to put females in contact, infant handling could enhance the probability for females to establish an emotional connection between them.

Chapter 3 – In this chapter, I focused on facial communication during playful interactions in geladas infants as a tool to understand the possible exchange of playful emotional states. Social play, especially in its most common and roughest form (i.e., play fighting), implies behavioral and emotional synchronization between players to maintain and share a playful mood and, therefore, to reduce the risk of escalation into real aggression (Aldis, 1975; Palagi, 2008, 2009). In geladas, playful contacts are the only positive interaction between different social groups (Dunbar and Dunbar, 1975). Such playful inter-group encounters - the so-called "play-units" - are carried out by infants (Dunbar and Dunbar, 1975; Palagi and Mancini, 2009). To evaluate the capacity of infants to communicate and maintain a playful mood during social play interactions, I investigated the role played by two facial expressions that are related to a positive emotional state - namely Play Face and Lip-Smacking - in wild immature geladas engaging in play fighting. More precisely, I focused on Rapid Facial Mimicry (RFM), i.e. the rapid replication (in less than 1 second) of a facial expression emitted by a subject in response to a facial expression emitted by another subject. Because RFM is considered one of the possible way through which emotional sharing and contagion – a basic form of empathy - can be put in place (de Waal and Preston, 2017), this investigation can contribute to a better understanding on the origin of emotional sharing in primates.

Chapter 4 – In this chapter I investigated another behavior that represents a promising tool to study socio-emotional communication: yawn contagion. This phenomenon consists in a subject yawning after having perceived the yawn emitted by another subject. Yawn contagion is considered a behavioral and possibly emotional matching based on the PAM (de Waal and Preston, 2017). Given that yawn contagion is linked to motor, and possibly emotional contagion (the basic layer of the Russian-doll model proposed by Preston and de Waal, 2002), its presence could be a good indicator of empathy-related phenomena.

Contrary to spontaneous yawning - which is widespread in vertebrates and therefore evolutionary ancient (Baenninger, 1987) - contagious yawning seems to be evolutionarily much more recent, as it has only been observed in a few species that are characterized or have been characterized in the past by a complex sociality (Demuru et al., 2022). Here, I investigated yawn contagion in the bonobos, the Hominini species that – along with chimpanzees - share with humans the last common ancestor.

Similar to geladas, although in a different way, in bonobos, adult females are influential and play a key role in determining group dynamics, with female dominance rank maintained via socio-sexual interactions, alliances and agonistic support (Parish, 1996; Furuichi, 2011). Contrary to other Hominini (chimpanzees and humans) in which yawn contagion has been consistently found, not all studies found yawn contagion in bonobos, with mixed results concerning the effect of familiarity and no replication on its modulating factors (Demuru and Palagi, 2012; Tan et al., 2017; but see Amici et al., 2014 for contrasting results). To address this puzzling issue, I explored the presence of yawn contagion in another group of bonobos. Because yawn contagion may underlie emotional contagion, defining whether bonobos show yawn contagion in different groups may add to the understanding on whether emotions could be shared in the last common ancestor between the humans and the *Pan* evolutionary line.

Chapter 5 – In the last chapter of my thesis, I moved to humans and I investigated the possible linkage between yawn contagion and socio-emotional attachment between pregnant women and babies. Mother-infant attachment is extremely important for the establishment of an adequate socio-emotional communication (Okabe et al., 2012) and it starts long before birth, also thanks to the hormonal changes caused by pregnancy (Napso et al., 2018; Barba-Müller et al., 2019; Tichelman et al., 2019). Here, I assessed whether yawn contagion is more frequent in pregnant versus nulliparous women, as the first cohort of subjects is in the process of developing the prenatal mother-infant bonding in preparation for maternal care that involves both hormonal and neurobiological changes in the neuronal circuitry related to attachment and empathy (Brandon et al. 2009; Barba-Müller et al. 2019; Tichelman et al. 2019; Palagi et al. 2020). Because it is debated whether yawn contagion is an involuntary phenomenon that may be influenced by socio-emotional attachment, this study may add a piece to our understanding of how emotions are non-verbally and implicitly transmitted to others.

Via the studies presented above, the aim of my doctoral research is to contribute – via a comparative approach - to the understanding of how socio-emotional communication may have emerged and is maintained in primates, including humans, especially when females and infants are involved.

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CHAPTER 1

Wild geladas (*Theropithecus gelada*) in crops - more than in pasture areas - reduce aggression and affiliation

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Wild geladas (*Theropithecus gelada*) in crops - more than in pasture areas - reduce aggression and affiliation

Marta Caselli[1], Anna Zanoli[1], Carlo Dagradi[1], Alessandro Gallo[1], Dereje Yazezew[2], Abebe Tadesse[2], Michele Capasso[3], Davide Ianniello[3], Laura Rinaldi[3], Elisabetta Palagi[4,5,*], Ivan Norscia[1,5,*]

[1]University of Torino, Department of Life Sciences and Systems Biology

[2]Debre Behran University, Department of Biology

[3]University of Napoli Federico II, Department of Veterinary Medicine and Animal Production

[4]University of Pisa, Department of Biology, Unit of Ethology

[5]University of Pisa, Natural History Museum

*Shared corresponding and senior authors: Ivan Norscia[1,5], Department of Life Sciences and Systems Biology, University of Torino, via Accademia Albertina 13, 10123, Torino, Italy. Email: ivan.norscia@unito.it Tel: +39 011 6704547

Elisabetta Palagi[4,5], Unit of Ethology, Department of Biology, University of Pisa, via Volta 6, 56126, Pisa, Italy. Email: elisabetta.palagi@unipi.it Tel: +39 050 2211385

Abstract

Human–primate interfaces are expanding and, despite recent studies on primates from peri-urban environments, little research exists on the impact of agriculture and/or pasture areas on primate social behavior and health. We assessed how crop/pasture areas potentially alter social behavior and health of wild geladas (*Theropithecus gelada*) frequenting the unprotected area of Kundi (Ethiopia). We predicted that compared to pasture areas, crop areas (i) would be more challenging for geladas (prediction 1) and (ii) would have a greater impact on both aggressive and affiliative behavior, by reducing grooming time and enhancing competition (prediction 2). During January–May 2019 and December 2019–February 2020, we collected data (via scan, focal animal sampling, and video analyses) on direct human disturbance, external signs of pathologies and social behavior of 140 individuals from 14 one-male units and two all-male units. Animals experienced the highest level of human disturbance in crop areas (in line with prediction 1). Individuals from the groups preferentially frequenting crop areas showed the highest prevalence of external signs of pathologies consistent with chemical and biological contamination (alopecia/ abnormally swollen parts). We collected 48 fecal samples. Samples from frequent crop users contained the highest rates of parasitic elements/gram (egg/larva/oocyst/cyst) from *Entamoeba histolytica/dispar*, a parasite common in human settlements of the Amhara region.

In crop areas, subjects spent less time grooming but engaged in lower rates of intense aggression (in partial agreement with prediction 2). We speculate that the reduction in social behavior may be a tactic adopted by geladas to minimize the likelihood of detection and maximize food intake while foraging in crops.

Keywords Primates, Behavioral change, Social behavior, Human impact, Primate health, Ethiopia

Introduction

The growing expansion of human settlement (Koh and Wilcove, 2008) is causing changes in wildlife behavior due to the forced coexistence of wildlife and humans (Sih et al., 2011). Previous investigations report behavioral changes in different taxa (reptiles: Batabyal et al., 2017; birds: Blumstein et al., 2005; mammals: Belton et al., 2018). Nonhuman primates (hereafter primates) are no exception and are particularly affected because approximately 30% of the existing species live in proximity to human settlements and rely on anthropic land cover for their maintenance activities (McLennan et al., 2017; Galán-Acedo et al., 2019).

Various types of human-primate interfaces, including tourist-provisioned sites, temples, urban settlements, and agricultural fields (Kaburu et al., 2019; Balasubramaniam et al, 2020; Jaman and Huffman, 2013), are described in literature. Agricultural areas can have a particularly strong impact on primate behavior (Arroyo-Rodríguez and Fahrig, 2014) because crops are often associated with close human settlements (Minta et al., 2018). They can include patches with clumped, high-quality and palatable resources, leadingto high-risk crop foraging by primates (Riley et al., 2013). Hill (2018) proposed two hypotheses to explain crop for- aging: the *crops as fallback foods hypothesis*, according to which primates would feed on crops when wild resources are scarce, and the *crop* *foraging as an optimizing strategy hypothesis*, according to which the high risk associated with crop foraging would be compensated by an increase in nutritional intake, with consequent benefits for reproductive potential.

One of the main risks that primates face when frequenting areas in which humans are present, including agricultural fields, is related to direct or indirect pathogen transmission among humans, livestock, and primates (Goldberg et al., 2007; Krief et al., 2010). Such transmission can include gastrointestinal parasites, such as protozoans in *Gorilla gorilla gorilla* (*Giardia intestinalis*; Sak et al., 2013), several nematode species in *Papio* spp. (Hahn et al., 2003), and, if wild or domestic canids are present, the cestode *Taenia serialis* in *Theropithecus gelada* and other primates (Schneider-Crease et al., 2017; Chanove et al., 2019).

The health of wild primates can also be impacted when their home ranges include agriculture land and herbicides and other chemical pollutants are used on crop fields (Garabrant and Philbert, 2002). For example, 2,4-dichlorophenoxyacetic acid, frequently used for weed control (de Castro Marcato et al., 2017), has been associated with the presence of alopecia (e.g. in dogs: Charles et al., 1996), tumors (in humans: Anthony and Saleh, 2013), and reproductive problems (e.g. in chimpanzees and olive baboons, Krief et al., 2017). We urgently need more evidence on the possible harm due to the ingestion of herbicides and pesticides.

Finally, different types of human-primate interfaces may variably influence primate social behavior. Chowdhury et al. (2020) found that in chacma baboons, *Papio ursinus*, social grooming decreased in anthropogenic areas. Other studies were mostly focused on macaques. For example, in peri-urban areas, *Macaca radiata* showed reduced grooming effort due to interaction with both visitors and local residents (Balasubramaniam et al., 2020). In temple areas, depending on the level of human–monkey interaction, *Macaca mulatta* can reduce social grooming considerably (Kaburu et al., 2019), but in urban areas they can increase grooming and play compared to rural areas (Jaman and Huffman, 2013). The time spent grooming in *Macaca fascicularis* varies depending on whether the interaction with humans is moderate or high (Marty et al., 2019).

The social behavior of primate groups frequenting agricultural lands may be particularly affected for at least three reasons. First, the measures used by humans to protect their crops, such as chasing, throwing objects, or even shooting at animals (Osborn and Hill, 2005), can disrupt primate behavior (McKinney, 2015; McLennan et al., 2017). Second, the high-quality, concentrated resources found in agricultural lands can lead to reduced affiliation and increased overt competition (Jaman and Huffman, 2013; Arseneau-Robar et al., 2016). Third, time budget trade-offs can come into play, as in agricultural areas primates might be constrained by time linked to a higher risk of being herded by humans that monitor them to keep them away (Priston et al., 2012; Chowdhury et al., 2020).

Based on this framework, our goal was to contribute to a better understanding of how different human–primate inter-faces can affect the health and social behavior of nonhumanprimates. Specifically, we investigated whether the relative use of two different human–primate interfaces, namely agriculture and pasture, affected the health and the social behavior of a population of wild geladas (*Theropithecus gelada*), a primate species endemic to Ethiopia. Geladas are group-living, terrestrial, and mostly herbivorous; consequently, part of their natural plant food species is shared with livestock (Fashing et al., 2014). Moreover, the products of cultivated plants (e.g., *Eragrostis tef*) are also highly attractive to geladas, which can approach human settlements and enter crop fields in search of food (Abu et al., 2018). Based on the observation that primates frequenting crops can be exposed to direct (e.g. active chasing: Osborn and Hill, 2005) and indirect human disturbance (e.g. chemical and biological sources of potential pathology: Garabrant and Philbert, 2002; Nunn et al., 2006), we predicted that the geladas using the crop area the most would be exposed to more frequent direct human disturbance (prediction 1a), higher risk of developing pathologies (prediction 1b), and increased risk of infection by parasites typical of human settlements (prediction 1c).

Geladas live in a multi-level society whose basic unit is the one-male/multi-female unit (hereafter, OMU) (Dunbar and Dunbar, 1975; Zinner et al., 2018). An OMU generally comprises one adult male, several adult females, and their offspring. Bachelor groups, separate from OMUs, are called all-male units (hereafter, AMU). OMUs and AMUs can form teams, bands and, at a larger level, herds, which can include hundreds of individuals (Dunbar and Dunbar, 1975; Snyder-Mackler et al., 2012; Zinner et al., 2018). High-intensity sporadic aggression is observed when a male tries to take over a group or to claim a territory (Beehner and Bergman, 2008). However, the absence of a strict reproductive season and the control of a single male over a group of females largely reduces inter-male competition over females (Dunbar and Dunbar, 1975). Moreover, groups are characterized by extremely high tolerance levels (Dunbar and Dunbar, 1975). As a result, gelada societies are characterized by low rates of inter- and intra-group (OMUs/AMUs) aggression and high levels of affiliative social grooming between group members (Dunbar and Dunbar, 1975; Mancini and Palagi, 2009). Because human interference and resource competition in primates can lead to decreased affiliation (Jaman and Huffman, 2013) and increased aggressive patterns (Arseneau-Robar et al., 2016; Thatcher et al., 2019), both of which can jeopardize group cohesion and social stability, we predicted that geladas would spend less time grooming (prediction 2a) and engage in aggression of higher intensity when in the crop area compared to the pasture area (prediction 2b).

Methods

Study site and subjects

This study was conducted with a population of wild geladas frequenting the Kundi plateau, in the Wof-Washa area (Ethiopia, Amhara region, N9°40.402' E39°45.060'; altitude(min–max): 3370–3592 m). We followed the subjects from January to May 2019 and from December 2019 to February 2020, spanning the dry and the beginning of the small rainy season (for further information see Appendix S1), on a daily basis, five days per week (excluding days with heavy rain or mist), from around 9:30 to 17:00 (for a total of 94 full days and a total of 658 h). We considered that the small rainy season (cf. Yazezew et al., 2020) had started when the rain set in for three consecutive days. The late dry and early wet periods - often including the post-harvesting phase- can be key periods of nutritional need, possibly associated with crop raiding by geladas searching for crop food remains and seeds (Hirvonen et al., 2016; Dunbar, 1977).

Surrounded by cliffs, the Kundi plateau (26 ha) is characterized by crop (about 12 ha) and pasture areas (about 14 ha), which have the same visibility conditions (Fig. S1).

In this study, we defined "crop area" as the agriculture fields (including human settlements) and the zone within 300 linear meters from the closest house or cultivated land. This criterion allowed for cultivated land, houses, domestic animal shelters, and passage zones from crop to crop or from crop to houses to be included in the “crop area.” We defined “pasture area” as the grassland without human settlements and cultivated fields, where livestock (horses, goats, sheep, donkeys, and cows) grazed during the day, led by shepherds. During the study period, animals spent 77.083±14.360 (mean±SE) and 276.458±23.500 (mean±SE) non-consecutive minutes per day in the crop and pasture areas, respectively. Gelada groups were free to move down the cliffs from the plateau. Further information on the study is available in Appendix S1.

In the first month of the study, a subset of groups frequenting the Kundi plateau were habituated and surveyed by four to six researchers (EP, IN, MaC, AZ, CD, AG). Group size, sex ratio, age ratio, and natural markers of the central male and/or other individuals (as detailed below) were used to identify gelada groups (one-male unit; OMU/all-male unit; AMU), based on Dunbar and Dunbar (1975) criteria. This process required around one month and was facilitated by video-recording of the groups. We were able to survey 14 OMUs and two AMUs and counted 27 adult males, 79 adult females, 60 subadult individuals, 35 juveniles, and 65 infants (31 late, 21 early, 13 black; further information on the population is available in Appendix S1). The number of groups present on the plateau on a daily basis was 8.706±0.950 (mean±SE).

Individual discrimination was achieved for 140 subjects (excluding infants) by considering long-lasting distinctive features (including sex, size, permanent scars, deformations, and particular shapes of the red chest area in adults; Dunbar and Dunbar, 1975). Such features were identified during field observations or via video recordings during and after the field data collection.

Field data collection

Each day four observers (MaC, AZ, CD, AG) went on the Kundi plateau and split into two groups to search for the gelada groups toward the top and the bottom of the plateau, respectively. The group composition of observers changed every week, following a rotation schedule. One observer (videographer) recorded the videos and the other assisted the videographer by vocally recording the ongoing activities and the subjects involved in the behavior. Not all of the identified gelada groups were present on the highland every day. Thus, on each day (after the end of the habituation period) data were collected on the visible and recognizable groups, giving priority to the less commonly observed groups when multiple groups were present to reduce observation imbalance and ensure sufficient data collection for all groups.

We conducted scan sampling (Altmann, 1974) live (not on video) at 10-min intervals on the recognized, visible groups present on the plateau each day. We gathered a mean of 304.357±SE 43.879 scans per group covering the whole daily observation period. Multiple groups could be present in a scan. Whenever possible, we recorded for the purpose of this study (i) group identity, (ii) GPS position based on the central male position (Garmin GPS Map 64), and (iii) the percentage of individuals foraging.

Data on direct human–gelada interactions (e.g., chasing animals, throwing stones, sticks; see table S1 for a detailed description, video MPEG-1) were collected via an all-occurrences sampling method (Altmann, 1974) to gather data on each possible episode.

On the recognizable groups, we also collected data via two video cameras (Panasonic HC-V180, full-HD, 50 fps, optical zoom 50x) for a total of 120 h of videos. We gathered a mean of 8.071±SE 1.336 video hours per group and a mean of 2.128±SE 0.198 video hours per subject, spreading the observational effort across morning and afternoon.

Grooming videos were collected via 10-min focal sampling (Altmann, 1974), with the focal subject being selected on the basis of the criteria explained above (giving priority to visible, recognizable, and less observed subjects). If the grooming continued, the recording went on until the end of the grooming session to allow analyses on grooming duration. This rule was applied to all dyads, and extra video duration (after 10 min) was considered only to calculate grooming duration (normalized as explained in the behavioral data section). The videos including grooming lasted on average 11.502±SE 0.686 min and involved 22 adult males (belonging to both OMUs and AMUs), 30 adult females, 5 immature males, and 2 immature females.

Owing to the tolerant nature of the study species, aggressive encounters are known to be infrequent (Bergman, 2010; Dunbar, 2014). Hence, data on aggressive events were collected via all-occurrences sampling (Altmann, 1974). Cameras were always kept on, on the clearly visible groups. While the videographer recorded the scene, the assistant would describe the aggressive event aloud to also gather data on what happened off-screen if necessary. At least three aggressive events per group were recorded, involving 23 adult males, 61 adult females, 29 immature males, and 10 immature females. The observed aggressions occurred to displace individuals from a foraging spot.

Health and disturbance data, and operational definitions

We calculated how frequently the OMUs+AMUs (N=16)were present in the crop area by considering the number of scans in which each group was inside the crop area normalized over the total scans per group. The group position was assessed via GPS coordinates, referring to the alpha-males.We then separated the groups into two categories ("frequent crop users" and "infrequent crop users"), depending on whether the frequencies fell above or below the median frequency of the proportion of scans per group recorded in crops (median=0.189; range=0.020–0.340; Table S2; Fig. S1).

Then, we considered the number of events of direct human disturbance (e.g., humans chasing geladas using stones, dogs, sticks, shooting; Table S1, Fig.

S2, video MPEG-1) for frequent and infrequent crop users, normalized over the total scans per group in each area (i.e., crop *vs*. pasture).

On the basis of photos and videos, the individuals (adults and immatures) were considered as bearing external signs of pathology when they showed at least one of the following external signs: abnormal swelling on trunk, limbs, and/or neck, probably related to *Taenia serialis* infection, as it has been found in other gelada populations (Ohsawa and Dunbar, 1984; Nguyen et al., 2015; Schneider-Crease et al., 2017); and alopecia, defined as hair loss either diffuse or patchy, in areas where the loss could not be caused by infant clinging (Fig. 1). The external signs of pathologies were considered for males and two categories of females (lactating and non-lactating) due to the effect that lactation can have on the immune system (Wang, 2016). Depending on the group they belonged to, individuals were assigned to either frequent or infrequent crop user groups. Descriptive statistics on the external signs of pathology are included in Appendix S1.

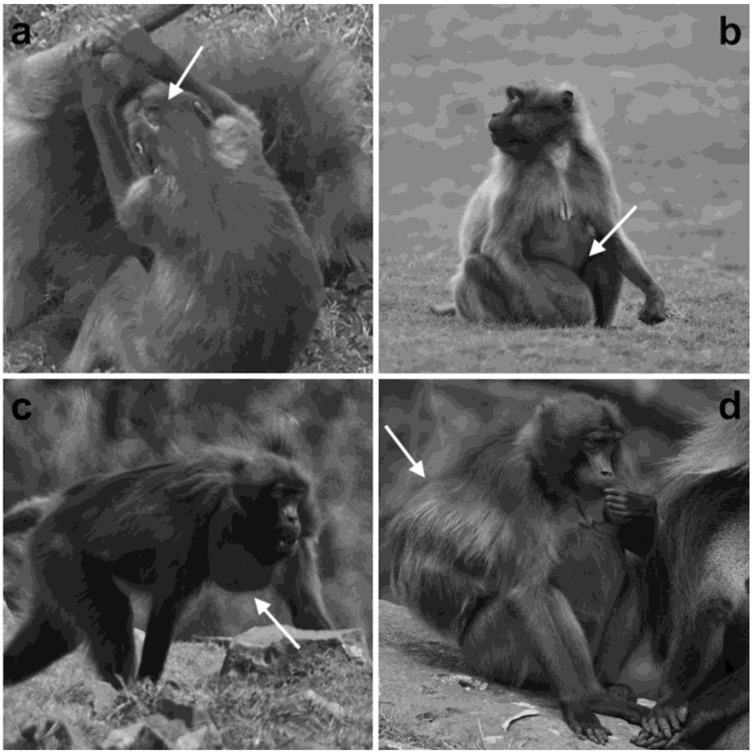

Figure 1 Pathologies observed in the geladas from the Kundi plateau: (a) adult female with alopecia, (b-c) adult female with abnormal swelling, (d) adult female with both alopecia and swelling. Photos by: Ivan Norscia, Alessandro Gallo, Carlo Dagradi

Behavioral data and operational definitions

We determined the daily frequency of foraging in the pasture and crop areas by considering the number of scans in which at least 10% of animals were foraging in either area normalized on the total number of daily scans per area.

Data on grooming were extracted from videos using the focal animal sampling (Altmann, 1974). To calculate grooming duration, we considered a grooming session as started when one of the two individuals began cleaning the fur of the other, and as finished when grooming was interrupted for at least 10 s (Mancini and Palagi, 2009). We recorded (i) groomer and grooming receiver identities, (ii) age class of both individuals (adult or immature), (iii) sex class (male or female), (iv) time spent grooming, and (v) area where grooming took place (pasture or crop). Because the observation time varied across dyads, for each dyad we divided the daily time spent grooming by the focal daily observation time of that dyad (normalized data).

The aggressive events were extracted from video- and audio-recorded information, following an all-occurrences method (Altmann, 1974) on the observable groups. For each aggressive event, we recorded the following data: (i) the identity of the aggressor (individual that initiated the first agonistic pattern) and the identity of the recipient (the individual that received the first aggressive pattern), (ii) age class (adult or immature), (iii) sex class (male or female), (iv) intensity of aggression, i.e. mild (chasing or chasing attempt without contact between opponents) or strong (chasing with contact between opponents, video MPEG-2), (v) whether aggression was intra- or inter- group, and (vi) the area where the aggression took place (pasture or crop). We recorded a total of 114 aggressive events, with a minimum of three aggressive events per group. All videos were analyzed via the free software VLC 3.0.6 (©VideoLAN) by MaC and AG (Cohen's value for inter-observer reliability calculated on 10% of the total grooming/aggressive events ≥ 0.75).

Fecal sample collection and parasitological analyses

We collected 48 fresh fecal samples (preserved in 10% formalin) from 48 unique individuals during observations and identified the samples as from individuals in the frequent or infrequent crop user group. The number of gastrointestinal parasitic elements (egg/larva/oocyst/cyst)/g of feces was determined using the FLOTAC pellet dual technique (Cringoli et al., 2010). This protocol is a multivalent, quali/ quantitative copromicroscopic method for detecting parasitic elements (eggs, larvae, oocysts, and cysts) in animal fecal samples, with an analytical sensitivity of one parasitic element per gram of feces (EPG/LPG/OPG/CPG). The pellet technique is performed for samples with unknown fecal material weight, so the weight of the fecal material can be obtained after weighing the sediment in the tube (pellet) after filtration and centrifugation of the fecal sample. These steps are very important for discriminating between parasites and pseudoparasites, considering that the identification of parasites in fecal samples is often complicated by the high fiber content of the animal diet, as well as the common presence of pollen, plant tissue, flowers, and invertebrate fragments (accidentally ingested with the plants), all of which can be misclassified as parasitic structures (Alvarado-Villa-lobos et al., 2017).

Two different flotation solutions were used to detect the gastrointestinal parasites: FS2 (sodium chloride solution, specific gravity=1200) and FS7 (zinc sulfate solution, specific gravity=1350). Different magnifications were used,

×100 and ×400, respectively, for the study of egg/larvae of helminths and cysts/oocysts of protozoa.

The diagnostic technique described above does not allow the identification at the species/assemblage level, so it was not possible to measure the specific richness.

Statistical analyses

Because of the small sample size (N<10: $N_{frequent\_OMU\_crop\_users}=8$, $N_{infrequent\_OMU\_crop\_users}=5$; not testable for normality), we employed a nonparametric Mann-Whitney test (SPSS 20.0) to compare the frequencies of direct human disturbance (Table S1) to primates between frequent and infrequent crop users. We included in the analyses the groups that underwent at least two disturbance events (Table S1, Fig. S2, video MPEG-1). We excluded three groups not meeting this condition. Exact values were selected following Mundry and Fischer (1998).

Owing to non-normal variable distribution (Kolmogorov–Smirnov test: $N_{days}=48$; $P<0.05$), we used the non-parametric paired Wilcoxon signed-rank test (Siegel and Castellan, 1988) to compare the daily frequency of foraging in crop and pasture areas. We applied a Monte Carlo randomization (10,000 permutations) (Bros and Cowell, 1987) to account for possible data pseudoreplication (same individuals present on different days).

We ran three generalized linear mixed models (GLMM) with three different target (dependent) variables, on three different aspects: presence of external signs of pathology ($GLMM_1$), grooming duration ($GLMM_2$), and aggression intensity ($GLMM_3$).

$GLMM_1$ was run to explore what individual features could affect the presence of external signs of pathology. We included in the model the occurrence of external signs of pathology as a dependent, binomial variable (factorial; presence/absence). We included four predictors as fixed factors: age class (factorial; adult/immature, excluding infants), sex class according to the presence of infants under lactation (factorial; non-lactating females; lactating females; males), group category based on the level of frequenting the crop area (factorial; frequent and infrequent crop users), and the group size (numeric). The group identity was included as a random factor.

To compare the parasite load (number of parasitic elements/g of feces) between frequent and infrequent crop users, we applied the exact Mann–Whitney nonparametric test (Mundry and Fischer, 1998; Siegel and Castellan, 1988; non-normal distributions; Kolmogorov–Smirnov test: N=48, *Ancylostomatidae* P=0.001; *Chilomastix* spp. P<0.001; *Entamoeba histolytica/dispar* P<0.001; *Endolimax nana* P=0.007; *Giardia intestinalis* P<0.001). The level of probability was adjusted according to the Bonferroni correction (α=0.010).

GLMM$_2$ was run to test the effect of area (crop/pasture) on the daily time spent grooming by dyads. We included the following predictors (factorial fixed factors): area where grooming took place (pasture/crop), season (dry/small rainy), age class of the two subjects involved in the grooming (adult/immature), sex class (male/female), crop use frequency (frequent/infrequent crop users), and group type (OMU/AMU). The grooming dyad and the unit identity were included as random factors.

Finally, GLMM$_3$ was run to investigate what variables could affect the intensity of aggression. Due to the small number of aggressive events involving AMU (N=2), for this analysis we considered only aggressive events involving OMUs. The model included the intensity of aggression as a binomial, dependent variable (mild/strong). We included the following fixed factors: area where the aggression took place (pasture/crop), season (dry/small rainy), dyad age class (same/different), dyad sex class (same/different), dyad group (inter-/intra-group aggression), and crop use frequency of both aggressor and recipient (frequent/infrequent crop users). The aggressor-recipient dyad and the OMU membership of individuals were included as random factors.

We fit all three models in R (R Core Team, 2018; version3.5.1) using the function “glmer” (in the case of binomial, dependent variable) of the R package *lme4* (Bates et al., 2015). We established the significance of the full model by comparison to a null model comprising only the random effects (Forstmeier and

Schielzeth, 2011). We used a likelihood ratio test (Dobson, 2002) 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 model using the R function "*drop1*" (Barr et al., 2013). For $GLMM_1$ and $GLMM_3$, the response variable was binary; hence we used a binomial error distribution. For $GLMM_2$, we $\log_{10}$-transformed the daily proportion of time spent grooming to reach a normal distribution after verifying the distribution and homogeneity of the residuals by the visual inspection of the qqplot and plotting the residuals against the fitted values (Estienne et al., 2017). For multinomial predictors with a significant main effect, we used a multiple contrast package (*multcomp*) to perform all pairwise comparisons for each bonding level with the Tukey test (Bretz et al., 2010). In this case, the level of probability was adjusted according to the Bonferroni correction. The effect size was calculated via the package "*effects*".

Results

Prediction 1: direct and indirect human disturbance

As concerns prediction 1a, we found that human direct disturbance was significantly more frequent for frequent crop users than for infrequent crop users (exact Mann–Whitney test: $N_{frequent\_crop\_users}=8$, $N_{infrequent\_crop\_users}=5$, U=4.000,

$Z=-2.342$, $P=0.019$).

Via $GLMM_1$, we tested the variables that potentially affected the presence of external signs of pathology (target variable; N=140 cases) (prediction 1b). The full model differed significantly from the null model (likelihood ratio test: $\chi^2=18.102$, $df=5$, $P=0.003$). There was a small to moderate but significant effect of the variable group category (frequent crop users/infrequent crop users; effect size=0.334; P=0.028; Table 1) and sex (effect size=0.398; P=0.016; Table 1) on the target variable. Moreover, a trend of significance was observed for the variable age (effect size=0.272; P=0.055; Table1). In particular, the prevalence of external signs of pathology was highest in the frequent crop users, and among adults it was lower in lactating females than in males and non-lactating females (Fig. 2a and b; Table1; Tukey test; non-lactating females vs. lactating females, Est=1.695; SE=0.587, P=0.011; lactating females vs. males, Est=−1.392, SE=0.576, P=0.041; non-lactating females vs. males, Est=0.304, SE=0.543, P=0.842).

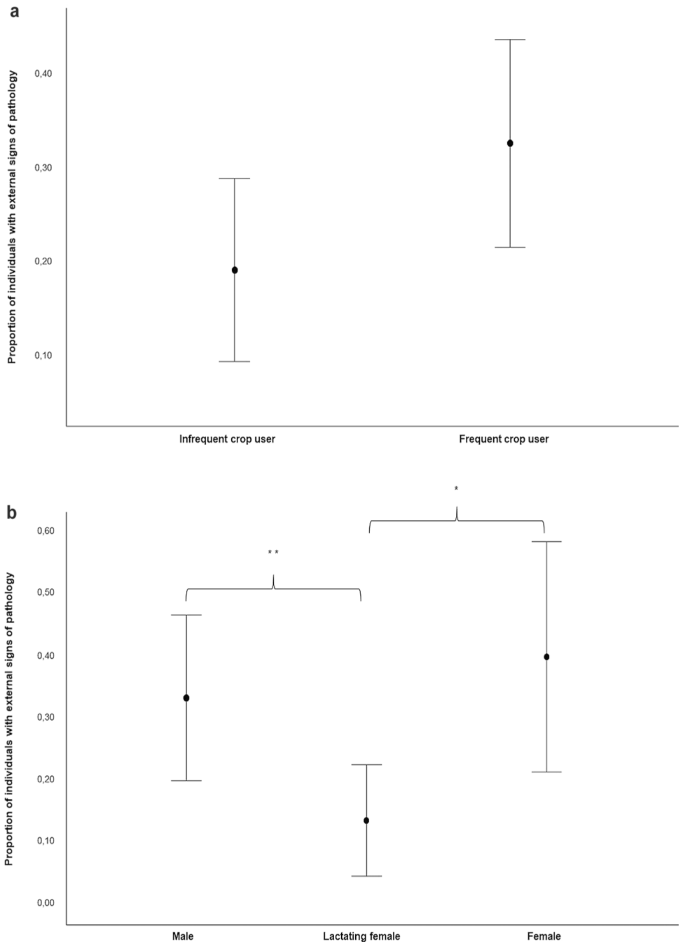

Figure 2 a Proportion of individuals with external signs of pathology in infrequent and frequent crop users (GLMM$_1$, N = 140, variable condition: $\chi^2 = 2.198$, $P = 0.028$; full results: Table 1); **b** proportion of individuals with external signs of pathology in non-lactating females, lactating females, and males (GLMM$_1$, N = 140, variable condition: $\chi^2 = -2.417$, $P = 0.016$; full results: Table 1). Mean (circle) and 95% confidence (bars) are indicated. *$P < 0.05$ and **$P < 0.01$

In the following analysis, we checked for differences in the number of parasitic elements/g found in the feces of frequent and infrequent crop users (prediction 1c). In the fecal samples of both frequent and infrequent crop users we found *Giardia intestinalis* (detected for the first time in a wild gelada population; mean±$\text{SE}_{\text{infrequent\_users}}$=1480.00±851.66; mean±$\text{SE}_{\text{frequent\_users}}$=386.38±198.37), *Ancylostomatidae* (mean±$\text{SE}_{\text{infrequent\_users}}$=231.45±63.75; mean±$\text{SE}_{\text{frequent\_users}}$=249.68±67.47), *Chilomastix* spp. (mean±$\text{SE}_{\text{infrequent\_users}}$=36.14±10.43; mean±$\text{SE}_{\text{frequent\_users}}$=30.32±19.08), *Endolimax nana* (mean±$\text{SE}_{\text{infrequent\_users}}$=22.21±6.05; mean±$\text{SE}_{\text{frequent\_users}}$=18.63±3.09), and *Entamoeba histolytica/dispar* (mean±$\text{SE}_{\text{infrequent\_users}}$=1.31±0.73; mean±$\text{SE}_{\text{frequent\_users}}$=21.47±12.99). We found that the number of parasitic elements/g of *Entamoeba histolytica/dispar* was significantly greater in frequent crop users compared to infrequent crop users (exact Mann–Whitney: $N_{\text{infrequent\_users}}$=29, $N_{\text{frequent\_users}}$=19, U=128.50, $P<0.001$). There was, however, no significant difference between frequent and infrequent crop users in the number of parasitic elements/g (i.e. egg/larva/oocyst/cyst) of *Ancylostomatidae*, *Chilomastix* spp., *Endolimax nana* or *Giardia intestinalis* (exact Mann–Whitney: $N_{\text{infrequent\_users}}$=29, $N_{\text{frequent\_users}}$=19; *Ancylostomatidae*: U = 262.00, P = 0.776; *Chilomastix* spp.: U=223.50, P=0.207; *Endolimax nana*: U=241.00, P=0.443; *Giardia intestinalis*: U=243.50; P=0.500).

Prediction 2: impact of crop and pasture areas on social behavior

Geladas foraged significantly less in the crop areas in comparison to pasture (Wilcoxon signed-rank test via Monte Carlo randomization: N_{days}=48,

$Z=-4.544$, $P<0.001$; $\text{mean}\pm\text{SE}_{\text{crop}}=0.306\pm0.058$; $\text{mean}\pm\text{SE}_{\text{pasture}}=0.760\pm0.035$).

In $GLMM_2$, we tested what variables potentially affected the time that the dyads spent grooming on a daily basis ($N_{\text{dyads}}=95$) (prediction 2a). The full model differed significantly from the null model (likelihood ratio test: $\chi^2=19.748$, $df=8$, $P=0.011$). Gelada dyads spent significantly more time grooming in the pasture than in the crop area (Fig. 3a; Table 1) and during the small rainy season than during the dry season (Table 1), with both variables showing a strong effect (absolute effect size > 1).

In $GLMM_3$, we tested what variables potentially affected the intensity of aggression ($N_{\text{aggressive\_events}}=114$) (prediction 2b). The full model differed significantly from the null model (likelihood ratio test: $\chi^2=15.723$, $df=6$, $P=0.015$). The variable area (crop/pasture) had a moderate to large significant main effect on agonistic encounters (effect size=0.616; Table 1). In particular, geladas engaged in more intense aggressive events when they were in the pasture area than when they were in the crop area (Fig. 3b; Table 1).

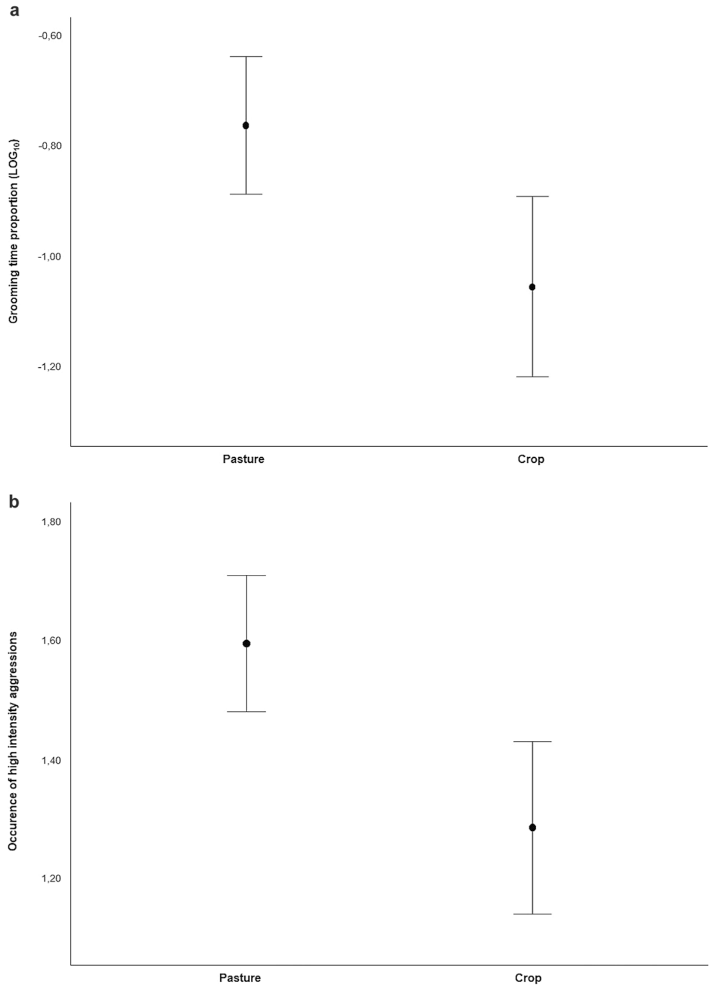

Figure 3 a Daily proportion of time spent in grooming interactions in pasture and crop area (GLMM2, N = 95, t value = −2.622, P = 0.010; full results: Table 1); **b** proportion of high-intensity aggression between pasture and crop area (GLMM3, N = 114, variable condition: χ^2 = −2.770, P = 0.006; full results: Table 1). Mean (circle) and 95% confidence (bars) are

Table 1 Results of GLMMs

Predictors	Estimates	SEM	CI_{95}	χ^2	*P*
GLMM$_1$ presence of external signs of pathology (N=140) (group identity was included as random factor)					
(Intercept)[a]	0.591	0.892	−1.156, 2.338	a	a
Sex (lactating females)[b]	−1.392	0.576	−2.520, −0.263	−2.417	**0.016**
Sex (non-lactating females)[b]	0.304	0.543	−0.761, 1.368	0.559	0.576
Age class (immature)[b]	−1.044	0.545	−2.112, 0.024	−1.915	0.055
Group_category (frequent crop users)[b]	1.189	0.541	0.129, 2.249	2.198	**0.028**
OMU size	−0.097	0.062	−0.217, 0.024	−1.573	0.116
GLMM$_2$ time spent daily in grooming interactions (N=95) (dyad and unit identity were included as random factors)					
(Intercept)[a]	−2.167	0.589	−3.321, −1.011	a	a
Sex_actor (female)[b]	−0.419	0.331	−1.067, 0.229	−1.265	0.210
Sex_receiver (female)[b]	−0.119	0.271	−0.651, 0.412	−0.440	0.662
Age class_actor (adult)[b]	0.538	0.445	−0.333, 1.410	1.210	0.230
Age class_receiver (adult)[b]	−0.224	0.313	−0.388, 0.837	0.717	0.482
Area (crop)[b]	−0.590	0.225	−1.031, −0.148	−2.622	**0.010**
Crop_users (frequent crop users)[b]	−0.377	0.256	−0.878, 0.124	−1.474	0.146
Group_type (AMU)[b]	0.116	0.468	−0.800, 1.032	0.248	0.805
Season (small rainy season)[b]	0.583	0.212	0.167, 0.999	2.047	**0.007**
GLMM$_3$ intensity of aggression (N=114) (dyad and OMU identity were included as random factors)					
(Intercept)[a]	0.512	0.564	−0.534, 1.557	a	a
OMU (inter-OMU)[b]	1.143	0.779	−0.383, 2.669	1.467	0.142
Sex_dyad (different sex)[b]	−0.017	0.486	−0.969, 0.935	−1.035	0.972
Area (crop)[b]	−1.478	0.534	−2.524, −0.432	−2.770	**0.006**
Age_dyad (different age)[b]	−0.588	0.470	−0.333, 1.508	1.251	0.211
Crop_user_dyad (different crop user	0.720	0.875	−2.435, 0.995	0.823	0.411

frequency)[b]					
Season (small rainy season)[b]	−0.731	0.541	−1.791, 0.329	−1.352	0.176

[a] Not shown as not having a meaningful interpretation

[b] These predictors were dummy-coded, with the reference categories as follows: $GLMM_1$-Sex: “male”; Age class: “adult”; Group_category: “infrequent crop users”; $GLMM_2$-Sex actor/receiver: “male”; Age class actor/receiver: “immature”; Area: “pasture”; Crop users: “infrequent crop users”; Group_type: “OMU”; Season: “dry season”; $GLMM_3$-OMU: “intra-OMU”; Sex_dyad: “same sex”; Area: “pasture”; Age dyad: “same age”; Crop user dyad: “same crop user frequency”; Season: “dry season”

Discussion

Direct and indirect human disturbance

Our results are consistent with the hypothesis that crop area can be challenging to wild geladas, because frequent crop users were more exposed to direct human disturbance (in line with prediction 1a) and a waterborne parasite (i.e., *Entamoeba histolytica/dispar*; in partial agreement with prediction 1c), and showed more signs of external pathologies (i.e., alopecia and abnormal swelling), in line with prediction 1b (Table 1; Fig. 2a).

According to previous studies on geladas and other primates, the observed external signs of pathology were compatible with the presence of ectoparasites (i.e., alopecia) or endoparasites (i.e., abnormal swelling) possibly shared with livestock and humans (Toft, 1986; Schneider-Crease et al., 2017). Throat swelling and alopecia may also be symptoms of iodine deficiency, which is common in the human population living in the Amhara region of Ethiopia, where this study took place (Abuye and Berhane,2007). These two pathology signs have also been observed in captive geladas (Borst et al., 1972). Similar symptoms may be caused by thyroid-disrupting chemical contaminants, including those used in agriculture (Maliszewska-Kordybach and Smreczak, 1998; Rolland, 2000). In particular, the 2,4-dichlorophenoxyacetic acid used in the study area as herbicide (pers. obs.) has been reported to cause tumors in humans (Anthony and Saleh, 2013) and alopecia in dogs (Charles et al., 1996). Currently there is a lack of information on whether iodine deficiency and the abovementioned herbicide are also responsible for external signs of pathology in geladas. Hence, the causality of alopecia remains unclear, whereas swelling is most likely the result of infection with *Taenia* spp. (*Taenia serialis* in wild geladas) reported for other gelada populations, with canids being the primary host of this tapeworm (Ohsawa and Dunbar, 1984; Nguyen et al., 2015; Schneider-Crease et al., 2017). In the study area, domestic dogs were present mostly around houses and crops, but both domestic and stray dogs may have roamed crop- and pastureland, which might explain why the area had only a small to medium effect on the presence of external signs of pathology. Even if we cannot confirm the presence of *Taenia serialis* in our study population from a biological point of view (to confirm the presence of this parasite, it is necessary to analyze urine samples; Schneider-Crease et al., 2017), the presence of abnormal swelling may be a predictor of the presence of this parasite. Indeed, *Taenia serialis* develops in the hypodermal musculature, causing abnormal swelling, and at the end of its development process the parasite perforates the skin and exits, causing suppurating masses (Ohsawa, 1979). Once all the mass is purged, the swelling disappears (Dunbar, 1980). On the other hand, the fact that apart from parasites, other factors specifically associated with farming may be linked to abnormal swelling and alopecia might explain why the effect of the area on the presence of external signs of pathologies was nevertheless significant. A diagnosis could not be performed on biological samples; therefore, none of these possibilities can be ruled out.

The fact that the external signs of pathology were significantly more frequent in non-lactating adult females than in lactating females (Fig. 2b) might be related to the immunological properties of oxytocin, produced during lactation to regulate milk production (Wang, 2016). On the contrary, testosterone in males can weaken the immune system, potentially explaining the more frequent signs of pathology in adult males than adult females (Roberts et al., 2004; Weisman et al., 2014; Muller, 2017). Another, nonexclusive explanation is that females with abnormal swelling may be in poorer health conditions and therefore less able to reproduce (Nguyen et al., 2015). The effect of sex, although significant, was small to moderate, possibly because various factors, together or separately, can cause alopecia and abnormal swelling (including parasites and chemical pollutants as described above).

The trend observed in the increase of the external signs of pathology in adults is in line with previous studies on geladas (Nguyen et al., 2015; Schneider-Crease et al., 2017). The higher frequency of these signs in adult than in immature subjects could be related to parasite accumulation and/or higher stress levels.

Adult subjects are more affected by social and environmental stress than immatures, causing a decrease in their immune system and making them more susceptible to parasitic infections (Muehlenbein and Bribiescas, 2005).

We also found the presence of a wide range of gastrointestinal parasites (Nematoda and Protozoa) in gelada fecal samples. Most of the parasites detected showed no differences between frequent and infrequent crop users. However, we found that *Entamoeba histolytica/dispar* was highest in the feces of the frequent crop users. This result may be linked to the especially high contamination levels by *E. histolytica* reported for the Amhara region around human settlements, compared to other regions of Ethiopia (Aiemjoy et al., 2017; Zemene and Shiferaw, 2018). In addition to indirect human disturbance (prevalence of external pathology signs and highest fecal parasite load), direct human disturbance was also high in the crop area. As a matter of fact, in the crop area, geladas were most likely to be chased away. This may have negative implications for gelada welfare. In other species, for example, it has been found that human-primate interactions (or even proximity) can be detrimental to health due to decreased feeding efficiency (related to increased vigilance for human aggression) and increased stress levels related to interactions with or threats by humans (Behie et al., 2010; Maréchal et al., 2011; Jaimez et al., 2012; Shutt et al., 2014; Chowdhury et al., 2020).

In summary, the first block of results suggests that agricultural activities close to human settlements can have a strong impact on wild gelada health. Frequenting agricultural areas may allow access to concentrated, high-quality resources (Strum, 1994; Osborn and Hill, 2005; Riley et al., 2013), but in the long term, crop foraging can have negative consequences on gelada health due to both direct and indirect disturbance. Further analyses on fecal samples collected from individuals showing external signs of pathologies could enable the identification of the possible direct link between the observed signs and parasite infections.

Differences in social behavior: crop versus pasture area

The time spent grooming was higher in the pasture than in the crop area (in line with prediction 2a; Table 1; Fig. 3a). However, contrary to our prediction 2b, aggressive events were more intense in the pasture than in the crop area (Table 1; Fig. 3b).

Relatively few studies have investigated how human primate interfaces can impact social relationships in primates, and the results of these studies are conflicting. For example, in contrast to our findings, studies on pygmy marmosets (*Cebuella pygmaea*: de la Torre et al., 2000) and on commensal macaque and baboon populations (Jaman and Huffman, 2013) revealed that groups living in close proximity to human villages spent more time grooming than the groups living in the countryside. On the other hand, other studies are consistent with our results. A previous report on *Macaca sylvanus* (Majolo et al., 2013) described a decrease in grooming inside tourist areas. In a population of bonnet macaques (*Macaca radiata*), the individuals that interacted more frequently with humans showed a greater tendency to monitor human activity and a decrease in grooming (Balasubramaniam et al., 2020). A recent study found that despite a positive relationship between the value of resources and the time spent in affiliative behavior, human interference had negative effects on grooming (Thatcher et al., 2019). The apparently divergent effects of human presence on social grooming may depend on the extent to which animals frequent anthropized areas, how far they are from human- monitored edges (e.g., Priston et al., 2012), whether they are regularly or occasionally exposed to human disturbance, and the type of disturbance. The fact that the area had a small though significant effect on the time spent grooming highlights the importance of grooming in geladas, because a certain level of this behavior is maintained in challenging locations (crop) as well. Indeed, grooming in geladas is used to preserve and reinforce social relationships (Mancini and Palagi, 2009), as in all the other cercopithecine species (Dunbar, 1991). Similar to previous reports on geladas and other primate species (Lee, 1984; Norscia et al., 2006; Yazezew et al., 2020), we found that the daily time spent grooming was higher in the rainy than in the dry season. During the dry season, food resources are normally distributed in more dispersed patches, and primates allocate more time to food search than to social interaction (Dunbar, 1992).

Acute anxiety due to transient challenging situations can lead to a reduction in social behavior, including both affiliation and aggression (Kalin and Shelton, 2003). The latter situation can apply to our study animals, which did not permanently live in proximity to human settlements: during the day, geladas came from the cliffs, entered the crop area to find better resources, acquired them from agricultural fields when possible, and then left. Indeed, geladas foraged significantly less in the crop than in the pasture area during the study period. This issue, along with other factors discussed below, can explain why in our study the aggressive events were less intense in the crop area, contrary to expectations. It has indeed been observed that the increased competition over high-value resources available in small patches can lead to increased conflicts in primate groups (*Macaca mulatta*: Southwick et al., 1976; *Papio anubis*: Wrangham, 1974; *Pan troglodytes*: Wittig and Boesch, 2003). However, stressful or threatening conditions can lead to behavioral suppression (Kalin and Shelton, 2003), also in the case of human presence (Maréchal et al., 2011; Muehlenbein et al., 2012). Behavioral suppression, including reduced aggression, can be a strategy to avoid conflicts when they are too dangerous (e.g., crowded conditions with limited possibility of escape) or to reduce detection probability (Judge and de Waal, 1993; le Roux et al., 2013). Considering that the study animals were not under crowded conditions (the groups frequenting the crop were not all present at the same time on the plateau), the second explanation is the most likely. Consistently, a previous study found that baboons (*Papio anubis*) can increase vigilance and reduce vocalizations to forage in crop fields (Warren, 2009). A previous study (le Roux et al., 2013) found that a concealing behavior is present in geladas, which show vocal suppression during extra-pair copulations in order to reduce the risk of potential aggression by the dominant male. Reducing social affiliation and aggression intensity may allow animals to focus on food provisioning, spend less time in the crop area (than in the pasture area), and decrease the probability of being detected. The area had a moderate to strong effect on aggression intensity, probably because of the importance of reducing risk while acquiring high-quality resources. Hence, when frequenting the area most exposed to human disturbance, geladas reduced their social behavior to possibly maximize provisioning and minimize detection risk.

In conclusion, this work provides a novel assessment of direct and indirect human impact on a wild population of *Theropithecus gelada* living in an unprotected area, in terms of both health status and social behavior. From a conservation point of view, our results highlight that in order to properly assess animal welfare in the wild, it is important to consider not only demographic data but also the impact that human activities can have on health and, importantly, on social interactions between subjects. Further parasitological analyses and seasonal data across the years and in different areas are necessary to fully clarify the repercussions of human disturbance on the welfare and social dynamics of wild geladas living in unprotected areas.

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Author contributions

Field training: EP, IN; Facilitation of fieldwork: DY, AT; Data collection: MaC, AZ, CD, AG; Conceptualization: IN, EP; Methodology: IN, EP, MaC; Data analyses: IN, MaC; Parasite analyses: DI, LR, MC; Writing and editing: IN, EP, MaC.

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Data availability statement

The study data are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest - The authors declare that they have no conflict of interest.

Ethical statement - This study is noninvasive and compliant with current Ethiopian and Italian law and University of Torino regulations, according to which no permit from the Bio-Ethical Committee was necessary.

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Appendix S1 – Additional information

Additional information on study site

Temperature and humidity were measured and recorded for every observation day (via Weather Station Thomson 513630). The minimum and maximum mean temperatures were 7.13 ± SE 0.13 °C and 12.46 ± SE 0.33 °C, respectively. The minimum and maximum mean humidity values were 72.88 ± SE 2.29% and 93.78 ± SE 0.93%, respectively. During the study months, seasonality on the Kundi plateau was characterized by the following periods: dry season (from December to March) and small rainy season (from April to May).

Crop areas mainly consisted in cultivations of cereal tef (*Eragrostis tef*) and small crops of potatoes (*Solanum tuberosum*). Pasture areas were dominated by graminaceous species, and particularly *Festuca* spp. The habitat falls into the Afro-alpine ecosystem and, except for cultivated plants, the only detected exotic species was *Eucaliptus* sp., on the cliffs. In both crop and pasture areas, cattle, sheep, goats, horses, and donkeys were present, but horses and donkeys usually stayed close to the human settlements. Dogs were also present in the crop area. Besides geladas, the medium-large, wild mammals present in the area included the primate *Colobus guereza*, the carnivore *Crocuta crocuta*, and the ungulates *Oreotragus oreotragus*, *Tragelaphus* spp., *Ourebia ourebi*. Other species are reported in the area but were not seen during our study (i.e., carnivores *Otocyon megalotis* and *Panthera pardus;* Yalden et al. 1996).

Additional information on study population

The mean number of individuals per OMU varied both for females and males. The mean number of males varied due to the presence of peripheral males in some OMUs: adult males = 1.286 ± SE 0.125, adult females = 5.643 ± SE 0.372, sub-adults = 4.214 ± SE 0.505, juveniles = 2.429 ± SE 0.552, infants = 4.643 ± SE 0.325 (late infants = 2.214 ± SE 0.334; early infants =1.500 ± SE 0.272; black infants = 0.929 SE ± 0.304). The mean number of adult males per AMU was 5.000 ± SE 2.000.

Additional information on pathological individuals

The mean number of individuals with external signs of pathology in infrequent or frequent crop user groups: i) adult males with swelling 0.375 ± SE 0.263 and 0.625 ± SE 0.263; ii) adult males with both alopecia and swelling 0.000 ± SE 0.000 and 0.125 ± SE 0.125; iii) adult females with alopecia 0.000 ± SE 0.000 and 0.125 ± SE 0.125; iv) adult females with swelling 0.625 ± SE 0.375 and 1.250 ± SE 0.628; v) adult females with both alopecia and swelling 0.250 ± SE 0.164 and 0.250 ± SE 0.164; vi) immatures with swelling 0.375 ± SE 0.183 and 0.625 ± SE 0.263 (the categories not reported did not present individuals with external signs of pathology). In total, 15% of individuals showed one or more of signs of external pathology.

Supplementary information - Figures

Figure S4

GPS point distribution of i) infrequent crop users in pasture (a) and crop (c) areas; ii) frequent crop users in pasture (b) and crop (d) areas. GPS points refer to both OMUs (referred to the alpha-male) and AMUs positions (referred to the male of the group closest to the observer). During the study period, 1697 GPS points were collected (1327 in the pasture area and 370 in the crop area).

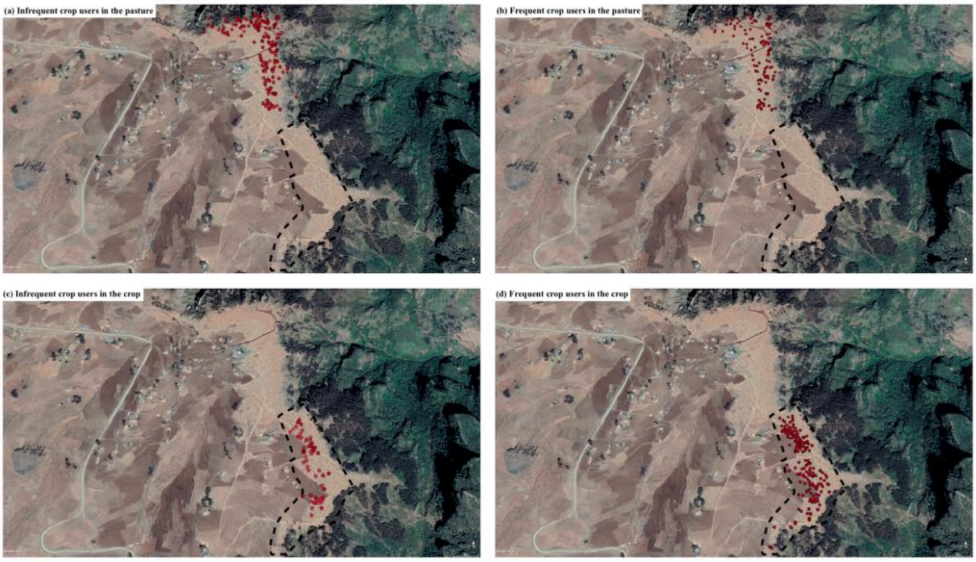

Figure *S*5

Rifle cartridge collected on the Kundi plateau, used to chase geladas away from crops

Supporting material - Tables

Table S1

Description of the different types of human disturbance observed/hypothesized during the study period. + = this type of human disturbance is more frequent in this area than in the other; - = this type of human disturbance is less frequent in this area than in the other.

Human disturbances	Description	Crop	Pasture	Direct	Indirect
Chasing	Humans drive off geladas by running after them	+	-	✓	
Throwing stones/sticks	Humans launch stones or use sticks to displace geladas	+	-	✓	
Livestock	Humans lead livestock towards geladas to displace them	-	+	✓	
Dogs	Humans use dogs to scare geladas away	+	-	✓	
Whip	Humans crack the whip to scare geladas	+	-	✓	
Shooting	Humans shoot to keep geladas away from crops	+	-	✓	
Parasites	Possible transmission of parasites between human/livestock and geladas suggested by certain parasites in geladas fecal samples (e.g *Entamoeaba histolityca* and *Giardia intestinalis*) and gelada abnormal swelling/alopecia (increased in crops)	+	-		✓
Chemical pollutants/agricultural substances	Observed use of detergents and 2,4D fertilizer in the study area. Possible health damages linked to the use of such substances	+	-		✓

Table S2

The table shows the frequency in the use of the crop area. Frequency was calculated by considering the number of scans in which each group was inside the crop area normalized over the total of scans/group. We separated the groups into two categories (‘frequent crop users’ and ‘infrequent crop users’), depending on whether the frequencies fell above or below the median frequency (median=0.189) of the proportion of scans/group recorded in crops.

OMU/AMU	Frequency of scan in the crop area	Classification
OMU1	0.147	Infrequent crop user
OMU2	0.016	Infrequent crop user
OMU3	0.306	Frequent crop user
OMU4	0.336	Frequent crop user
OMU5	0.185	Infrequent crop user
OMU6	0.164	Infrequent crop user
OMU7	0.285	Frequent crop user
OMU8	0.177	Infrequent crop user
OMU9	0.108	Infrequent crop user
OMU10	0.264	Frequent crop user
OMU11	0.286	Frequent crop user
OMU12	0.193	Frequent crop user
OMU13	0.298	Frequent crop user
OMU14	0.184	Infrequent crop user
AMU1	0.554	Frequent crop user
AMU2	0.000	Infrequent crop user

Supporting material - Video legends

MPEG-1: Human chasing a group of geladas with a stick

https://link.springer.com/article/10.1007/s10329-021-00916-8

MPEG-2: Inter-OMU aggression in the pasture area.

https://link.springer.com/article/10.1007/s10329-021-00916-8

CHAPTER 2

Infant handling increases grooming towards mothers in wild geladas (*Theropithecus gelada*)

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Infant handling increases grooming towards mothers in wild geladas (*Theropithecus gelada*)

Marta Caselli[a], Anna Zanoli[a], Elisabetta Palagi[b,c*], Ivan Norscia[a,b*]

[a] Department of Life Sciences and Systems Biology, University of Torino, Via Accademia Albertina 13, 10123 Torino (Italy)

[b] Natural History Museum, University of Pisa, Via Roma 79, 56011 Calci (Pisa, Italy)

[c] Unit of Ethology, Department of Biology, University of Pisa, Via Alessandro Volta 6, 56126 Pisa (Italy)

* Ivan Norscia and Elisabetta Palagi share the senior authorship

Corresponding authors: ivan.norscia@unito.it; elisabetta.palagi@unipi.it

Ivan Norscia ORCID: 0000-0002-1618-7717

Elisabetta Palagi ORCID: 0000-0002-2038-4596

Abstract

Infant handling – involving affiliative behavior from non-mothers to infants – is a phenomenon that is variably present in Old World monkeys and can be granted by mothers to obtain social services, such as grooming. Here we investigated for the first time whether infant handling could influence grooming exchange in wild geladas. We gathered data on the population of Kundi highland (Ethiopia) in 2019/2020. Via sampling on 15 focal mothers from eight different One-Male Units, we video-recorded 55 grooming sessions between focal mothers and non-focal females (mothers or non-mothers). We also recorded the possible occurrence of infant handling performed by non-focal females. We found that grooming sessions were longer between mother and non-mothers and in the presence than in the absence of infant handling. Hence, our results show that infant handling can influence the grooming exchange between wild gelada females. Because grooming is used to establish and reinforce social bonds in primates, infant handling may act as a 'social bridge' in a female bonded society. From an evolutionary perspective, infant handling strategies might represent the stepping-stone to more complex forms of infant care, such as allomaternal care and cooperative breeding.

Keywords: Female bonded societies, Grooming exchange, Infant manipulation, Infant handling, *Theropithecus gelada*

Introduction

In primates, social interactions between dependent infants and non- mother subjects might be a precursor of advanced shared offspring care, such as allomaternal care (Hrdy, 2009; Preston, 2013). In its most complex form (cooperative breeding), allomaternal care consists of prolonged and/or regular affiliative interactions between the infant and non-mothers, which are beneficial to the infant (e.g., increased survival; Solomon and French, 1997; Burkart et al., 2009; Hrdy, 2009; Tecot and Baden, 2015; Rosenbaum and Gettler, 2018). Such interactions can include babysitting, protective carrying, nursing, crèeching, or huddling for thermoregulation (Tecot and Baden, 2015; Dunayer and Berman, 2018). While infant care from the father is related to direct fitness benefits as it increases the chance of infant's survival (Fernandez-Duque et al., 2009), several hypotheses have been put forth to explain allomaternal care from others, which may produce indirect fitness benefits.

Allomaternal behavior may: i) be a consequence of the selection for high responsiveness to infants (*by-product hypothesis*; Quiatt, 1979); ii) provide indirect fitness benefits when the caretaker is related to the mother (*kin selection hypothesis*: Hrdy, 2009); iii) allow the acquisition of competence in maternal behavior (*learning-to-mother hypothesis*: Lancaster, 1971); iv) enhance agonistic support (*alliance formation hypothesis:* Maestripieri, 1994); v) lead to future care for own infants (the *reciprocity hypothesis:* Hrdy, 1976). An important consequence of allomaternal care is that it influences social dynamics as a result of increased tolerance, social attention, food sharing and other cooperative tasks (Snowdon and Cronin, 2007; Kramer, 2010; Burkart and van Schaik, 2010, 2016; Guerreiro Martins et al., 2019).

New World Monkeys (Callitrichidae and Pitheciidae species) and modern humans show complex forms of allomaternal care (involving different helpers participating in the process; Garber, 1997; Hrdy, 2009), whereas other primates may show more basic interactions between infants and non-mothers, such as infant handling. Although the literature is not always clear on the distinction between infant handling and allomaternal care - as compared to proper and regular allomaternal care - infant handling involves occasional affiliative behaviors such as nuzzling, play, a brief embracing, grooming, carrying and holding, and it does not provide discernible benefits to either the mother or the infant (Dunayer and Berman, 2018). Another difference between allomaternal care and infant handling can be that in the former, the care itself (rather than grooming) represents the payoff for infant access by non-mothers (Hrdy, 2009).

Great apes infrequently show infant handling (Mitani and Watts, 1997), which involves very occasional affiliative behaviors such as grooming, carrying and holding mainly performed by related females (Boose et al., 2018; Grueter et al., 2019). In Old World monkeys, infant handling is a variable phenomenon that can be performed by both females and males (Maestripieri, 1994; Paul et al., 2000). As regards male-infant interactions, several hypotheses have been proposed to explain infant handling, such as the *infanticide protection hypothesis* (with the male protecting their putative offspring from being killed by other males; Paul et al., 2000), and the *agonistic buffering hypothesis* (where males use infants as a shield to protect themselves against the attacks from high-ranking males; geladas, *Theropithecus gelada*: Dunbar, 1984; hamadryas baboons, *Papio hamadryas*: Kummer, 1967; *Papio* spp.: Smith and Whitten, 1988). The expression of infant handling by non-mother females is related to the species-typical social structure, with particular reference to social relationships among females (Maestripieri, 1994). Compared to more despotic species (e.g., Japanese macaques, *Macaca fuscata*: Schino et al., 2003; chacma baboons, *Papio ursinus*: Henzi and Barrett, 2002), mothers living in tolerant societies are more permissive and inclined to allow other females to manipulate their own offspring (Maestripieri, 1994; Ciani et al., 2012). Within biological markets (*sensu* Noë and Hammerstein, 1994, 1995), infant access can be granted by mothers in exchange for social services (e.g., for sex in snub-nosed monkeys, *Rhinopithecus roxellana*: Yu et al., 2013; for embracing in spider monkeys, *Ateles geoffroyi*: Slater et al., 2007; for grooming in tufted capuchins monkeys, *Sapajus apella*; Tiddi et al., 2010; in macaques, *Macaca* spp.: Gumert, 2007; Jiang et al., 2019), with mothers and non-mothers being considered as two trading classes exchanging different commodities (Henzi and Barrett, 2002; Tiddi et al., 2010). Such exchange follows the supply-demand law (Noë, 2001). Within the infant-handling domain, the ratio between supply and demand can be determined by the number of available infants. For example, in spider monkeys (Slater et al., 2007), the number of infants can be positively correlated with the proportion of embraces followed by infant handling. In long-tailed macaques (Gumert, 2007) and chacma baboons (Henzi and Barrett, 2002), the amount of grooming received by the mothers can be negatively affected by the increased number of available infants. Grooming is a valuable commodity that in non-human primates is used to establish, maintain, and restore social relationships (Dunbar, 1991). Non-mother females are attracted to newborn infants (Altmann, 1980; Maestripieri, 1994) and try to manipulate them around grooming sessions with the mothers (Muroyama, 1994; Henzi and Barrett, 2002; Gumert, 2007; Frank and Silk, 2009; Jiang et al., 2019). Hence, in female-bonded societies, the grooming exchanged between females can largely determine their social dynamics, and infant handling can influence the exchange of grooming, especially between mothers and non-mothers (e.g., Henzi and Barrett, 1999; Frank and Silk, 2009; Jiang et al., 2019). Consequently, infant handling could be a tool to maintain and improve female-female cooperative relationships, as it occurs in capuchin monkeys (*Cebus capucinus*), where females were found to handle the infants of the mothers with which they exchanged grooming and formed coalitions more frequently (Manson, 1999).

The aim of the present study is to investigate for the first time the possible linkage between infant handling and grooming in wild gelada females (*Theropithecus gelada*), a tolerant monkey species endemic to Ethiopian highlands (Dunbar and Dunbar, 1975). Geladas live in a multi-level society whose basic unit is represented by the One-Male Unit (OMU), usually composed of one adult male, several adult females with offspring and characterized by female philopatry and male dispersal (Kawai et al., 1983; Le Roux et al., 2011; Snyder-Mackler et al., 2012). Due to the female philopatry, gelada females are strictly related and show maternally inherited dominance status, even though overt aggressions are rare, and hierarchy is less steep than that observed in the baboon species (Le Roux et al., 2011). Female social cohesion is maintained via several positive interactions (social play: Mancini and Palagi, 2009; Gallo et al., 2021; agonistic support: Pallante et al., 2016; embracing: Pallante et al., 2019), including a large amount of grooming, exchanged only between females belonging to the same OMU (Dunbar and Dunbar, 1975; Dunbar, 1983, 2014). Thus, geladas are a suitable species to be considered to expand the existing literature on infant handling and its relation to grooming dynamics between females to geladas. Based on the above framework, we formulated the following predictions:

Prediction 1 - Females of different primate species can be interested in others' infants (Altmann, 1980; Nicolson, 1987), making mothers more attractive than non-mothers (Hrdy, 1976; Maestripieri, 1994). Non-mothers can be particularly motivated to groom females when they have an infant (patas monkeys, *Erythrocebus patas*: Muroyama, 1994; Tibetan macaques: Jiang et al., 2019; snubbed nosed monkeys: Yu et al., 2013; olive baboons: Frank and Silk, 2009). Hence, we predicted that mothers would receive more grooming than non-mothers (Prediction 1a). However, grooming sessions can be shorter in the presence than in the absence of infant handling because non-mothers may stop grooming mothers as they obtain access to the infant (Henzi and Barrett, 2002). Thus, we predicted that grooming sessions could be shorter in the presence than in the absence of infant handling (Prediction 1b). Still, previous literature indicates that mothers can receive more grooming than they give when they allow the infant manipulation to non-mothers (e.g., Tibetan macaques: Jiang et al., 2019; olive baboons: Frank and Silk, 2009). Consequently, we predicted that mothers would receive a larger amount of grooming by non-mothers in presence rather than in the absence of infant handling (Prediction 1c).

Prediction 2 - In other monkey species, according to the supply-demand law, the amount of grooming received by mothers decreases as the number of infants in the group increases (e.g., chacma baboons: Henzi and Barrett, 2002; long-tail macaques: Gumert, 2007). If a similar situation also applies to geladas, we expect to find a significant effect of infant availability on the grooming received by the mothers from the non-mothers (Prediction 2a). Moreover, when infants are used as a ‘commodity’ their age can make a difference because newborns are more attractive to other females and elicit more grooming than less dependent infants (Nicolson, 1987). Thus, if also in geladas infants are used as a ‘commodity’, we expect that newborns, more than older infants, could enhance grooming between mothers and non-mothers (Prediction 2b).

Prediction 3 - With exceptions (Muroyama, 1994; Jiang et al., 2019), previous studies showed that grooming is usually given to mothers by non-mothers before they are allowed to access the infant (e.g., chacma baboons: Henzi and Barrett, 2002; snub-nosed monkeys, Yu et al., 2013; tufted capuchin monkeys, Tiddi et al., 2010). Thus, we expected that mothers would obtain grooming preferentially before (rather than after) infant handling.

Methods

Study site, subjects and data collection

This study was conducted from January to May 2019 and from December 2019 to February 2020 on a wild gelada population (*Theropithecus gelada*) frequenting the Kundi plateau (Wof-Washa area, Amhara region, Ethiopia, N9◦40.402′ E39◦45.060′). Daily observations occurred from around 9:30–17:00 when the animals - which came from the cliffs in the early morning and left the cliffs in the evening - frequented the highland for grazing. During the first month of fieldwork, four observers (two authors and two field assistants) were able to count 21 groups based on size, sex- and age-ratio of each group and to particular signs of some subjects (Caselli et al., 2021). For this study, we focused on the seven most frequently seen OMUs, in which all adult females were identified. For each OMU, the number of infants was also known (Table 1). The infants' age-classes were defined as follows: black infants (from 0 to 6 months: the fur is black or dark brown), early infants (from 6 months to 1 year: the fur is brown, carried dorsally by the mother) and late infant (from 1 to 2 years: the fur is brown as an adult) (Kawai et al., 1983).

Table 1 Composition of the 7 OMUs involved in the study, including adult females (8.000 ± 0.690 mean ± SE), subadult females (1.71± 0.522 mean ± SE), juvenile females (0.857 ± 0.404 mean ± SE), late infants (2.143 ± 0.459 mean ± SE), early infants (1.000 ± 0.488), and black infants (1.286 ± 0.359 mean ± SE).

OMU	Adult females	Subadult females	Juvenile females	Infants	Late infants	Early infants	Black infants
OMU 1	11	1	2	6	3	2	1
OMU 2	6	3	0	5	0	2	3
OMU 3	8	1	2	4	3	0	1
OMU 4	9	1	0	6	3	3	0
OMU 5	6	0	0	3	2	0	1
OMU 6	7	4	2	3	1	0	2
OMU 7	9	2	0	4	3	0	1

Females were identified on the basis of the OMU identity, presence of dependent offspring and long-lasting distinctive features (size, permanent scars, alopecia, abnormal swelling of body parts, shapes of the red chest area). Owing to the presence of infants, lactating females (hereafter, 'mothers') were easier to identify than other females, and the identification was possible during field data collection. Non-lactating females (hereafter, ‘non-mothers’) were identified either on the field or via videos.

Data gathering was carried out using focal animal sampling (Altmann, 1974) on the identified mothers when visible. The mothers were video recorded (via Panasonic HC-V180 Full HD optic-zoom 50x, 2 s accuracy), and focal videos lasted around 10-min. In the end, for subsequent analyzes, we selected 15 mothers (hereafter ‘focal mothers’) with at least two grooming sessions involving another identified female (hereafter, ‘non-focal female’). From the total dataset of 120 video hours collected, we were able to analyze 7.21 video hours due to the proper visibility requirements. The selected video included 55 grooming sessions that involved the 15 focal mothers and 36 non-focal females interacting with the focal mothers (31 adult and subadult/juvenile non-mothers and 5 mothers; $N_{grooming\_session\_focal\_mother/another\_mother}=10$; $N_{grooming\_session\_focal\_mother/non\text{-}mother}=45$).

Video analysis and operational definitions

The video analysis was carried out via BORIS 7.9.24 (Friard and Gamba, 2016; https://www.boris.unito.it/), a free, open-source and multiplatform program that allows a user-specific coding environment to be set for a computer-based review of previously recorded videos. Once established the behavioral set (grooming given/received and infant handling), the coding was performed by MC using previously assigned keys. Subsequently, we extracted the exact duration of grooming (total and given/received by the focal mothers), of infant handling and the behavioral sequence string for each grooming session. The strings consisted of the code of different behaviors separated by a break symbol in the order as they occurred.

For each grooming session, we recorded: i) identity of the focal mother and the other interacting female; ii) OMU identity; iii) the status of the non-focal interacting female (mother/non-mother); iii) total duration; iv) duration of the grooming received and given by the focal mother; v) presence/absence of infant manipulation by the non-focal female (hereafter ‘infant handling’). We could not consider the dominance relationship between females because aggressive events were rare (only 12 aggressive events between females of the same OMU occurred during the entire study period, including threat displays). Due to their philopatry, the females within each OMU are highly related (Le Roux et al., 2011), even though their exact kinship relations are unknown.

Although infant handling may include aggressive behaviors (e.g., biting, pulling, hitting; Boose et al., 2018), we never observed aggressive behaviors toward infants during the study period. Moreover, infant carrying by non-mother subjects was never observed. We, therefore, considered only the following affiliative patterns as part of infant handling, occurring when the infant was in contact or proximity (within arm’s reach) with the mother: embrace, lift and nuzzle infant rear, play, retrieve and grooming (Dunbar and Dunbar, 1975;

Table 2, infant handling via grooming and retrieve is shown in Video S1). In only 1% of cases, we observed infant handling occurring outside the grooming context.

Table 2 Description of the behaviors considered as infant handling during the study. The behavioral items were extracted from the ethogram of Dunbar & Dunbar (1975).

Behavior	Description
Embrace	The handler holds the infant closely with one or both arms
Lift and nuzzle infant rear	The handler moves up and smells the rear of the infant
Play	The handler performs some playful actions with the infant
Retrieve	The handler blocks the infant to avoid its flight
Grooming	The handler inspects and cleans the fur of the infant

To calculate the grooming duration, we considered a grooming session as initiated when one of the two individuals (individual A: the groomer) began cleaning the fur of the other (individual B: the groomee), and as finished when grooming was interrupted for at least 13s. We considered this time window, which is larger than the mean duration of infant handling (12.292s±4.827 SE), to reduce the probability to consider one grooming session as two separated sessions interrupted by an infant handling event. We associated infant handling events with a specific grooming session when non-focal females handled the infant within 10s before the onset and/or after the grooming session. If infant handling occurred during the grooming session, we excluded the duration of infant handling from the duration of the grooming session. We collected N=17 grooming sessions with infant handling and N=38 grooming sessions without infant handling.

We calculated the proportion of grooming received by the focal mother per session as the duration (s) of the grooming received by the focal mother normalized over the grooming session's total duration. We calculated the proportion of grooming received by the focal mother across sessions by summing up the duration of the grooming received by the focal mother in all sessions normalized over the total time that the focal mother spent grooming.

Statistics

We ran a Linear Mixed Model (LMM) to test if the duration of a grooming session between females was influenced by the type of grooming dyad (grooming between the focal mother and another mother or between the focal mother and a non-mother), the presence of infant handling performed by the non-focal female, the number of in- group infants and the age class of the infant. In the model, we included the $\log_{10}$-transformed duration of the grooming session as a dependent variable (the transformation allowed the achievement of the homogeneity and the normal distribution of the residual, evaluated by looking at the qqplot and plotting the fitted values against the residuals; Estienne et al., 2017). We included as fixedd factors the grooming dyad type (binomial: dyad "focal mother/non-focal mother" or dyad "focal mother/non-mother"), presence/absence of infant handling (binomial), the number of in-group infants (numeric) and the age class of the infant (trinomial). The dyad identity and the OMU identity were included as random factors. We fit the LMMs in R (R Core Team, 2018; version 3.5.1) by using the function "lmer" of the R-package *lme4* (Bates et al., 2015).

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, 2002) 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 model by using the R-function "*drop1*" (Barr et al., 2013). We used a multiple contrast package (*multcomp*) to perform all pairwise comparisons for each bonding level with the Tukey test, when a multinomial predictor had a significant main effect (Bretz et al. 2010). The level of probability reported was adjusted according to the Bonferroni correction. The effect size was calculated via the R-package "*effects*".

In addition, we used the behavioral strings extracted via BORIS7.9.24 (see above) from each grooming session ($N_{behavioral\_strings}=N_{grooming\_sessions}=55$) to carry out a sequential analysis at the session level to assess the probability of temporal association between infant handling performed by the non-focal female and the grooming given and received by the focal mother. Via the freeware Behatrix 0.9.11 (Friard and Gamba, 2020), we ran a permutation test on the behavioral transition counts (‘Run random permutation test’ Behatrix function, 10,000 permutation test). Based on this, we generated a flow diagram of behavior-to-behavior transitions (including only the significant transitions).

In the subsequent analysis, we focused on the dyads composed by the focal mother and a non-mother. We compared the proportion of grooming received/session (seconds) between focal mothers and non-mothers (non-normal distribution; Kolmogorov-Smirnov test: $N_{grooming\_sessions}=45$; $P<0.001$) via the non-parametric, paired Wilcoxon Signed Rank test (Siegel and Castellan, 1988). Because the same female could be involved in more than one session, to account for data pseudoreplicaton we applied a Monte Carlo randomization (Bros and Cowell, 1987). To verify the presence of a possible correlation between the grooming received by the mothers from the non-mothers and the number of in-group infants, we ran a correlation via randomization test. The correlation test was employed with a number of 10,000 permutations by using the software Resampling Procedures 1.3 (David C. Howell, freeware).

Then, we focused on the focal mothers ($N_{focal\_mothers}=8$) that had been involved in at least one grooming session with infant handling and one grooming session without infant handling ($N_{grooming\_session}=11$; $N_{grooming\_session}=21$ considered for this analysis). Due to the small sample size ($N_{focal\_mothers}=8$; not testable for normality because $N<10$), we employed a non-parametric Wilcoxon

Signed Rank test to compare the proportion of grooming received by focal mothers across sessions in presence and in absence of infant handling performed by non-mothers. The eight females excluded from this analysis were involved in 6 grooming sessions with infant handling and 17 grooming sessions without infant handling.

Non-parametric tests and randomization procedures were carried out via SPSS 26.0. The threshold of statistical significance for all tests was set at $\alpha=0.05$.

Results

Prediction 1 and Prediction 2

Via LMM we tested whether grooming duration (target variable) was influenced by the type of the dyad involved (focal mother/another mother or focal mother/non-mother), the presence/absence of infant handling performed by non-focal females, the number of in-group infants and their age class. The full model significantly differed from the null model (likelihood ratio test: $N_{grooming\_sessions}=55$, $\chi^2=14.452$, $df=5$, $P=0.013$).

Prediction 1a – According to the LMM results (Table 3), grooming sessions were significantly longer when they involved the focal mother and a non-mother (P=0.014) compared to the other condition (grooming between mothers, Fig. 1; effect size>1).

Table 3 Full results of the LMM on the $\log_{10}$ transformed duration of grooming sessions ($N_{grooming\_sessions}$=55). Fixed factors: infant handling (presence or absence), dyad type (focal mother/non-focal mother or focal mother/non-mother), number of in-group infants and age class of infant (black infant, early infant and late infant). Identity and OMU membership were included as random factors.

LMM on $\log_{10}$ transformed grooming duration				
Predictors	Estimates	SEM	Chi-squared	P
(Intercept)[a]	2.288	0.292	a	a
Infant handling (yes)	0.232	0.106	2.187	0.033
Dyad type (focal mother/non focal mother)	-0.343	0.135	-2.543	0.014
Number of in-group infant	-0.076	0.067	-1.148	0.276
Infant age-class (early)	0.097	0.176	0.547	0.587
Infant age-class (late)	0.274	0.139	1.968	0.054

[a] Not shown as not having a meaningful interpretation.

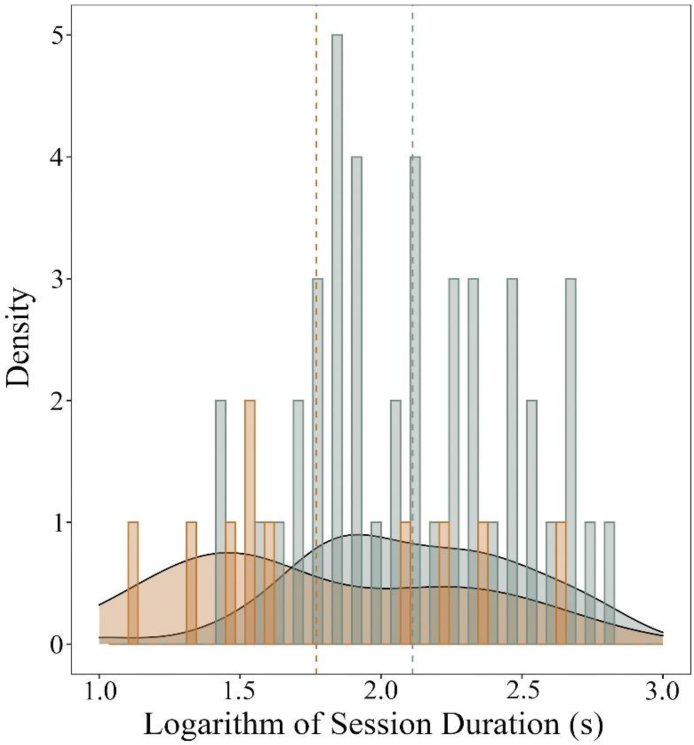

Figure 1 Bar plot combined with a density plot showing that duration of grooming sessions ($\log_{10}$ transformed data) is higher in focal mother/non-focal mother (orange bars) than focal mother/non-mother (grey bars) dyads (LMM: $N_{grooming\_sessions}$=55, P=0.036). The distribution of the duration of grooming sessions in focal mother/non-focal mother dyads and in focal mother/non-mother dyads is represented by the orange and grey density curves, respectively. Dashed line indicates the mean grooming duration. Each bar indicates a grooming session.

Moreover, when considering the grooming sessions that occurred between the focal mother and a non-mother, the proportion of grooming received/session was higher in the focal mother than in the non-mother (Wilcoxon Signed Rank via Monte Carlo randomization; $N_{grooming\_sessions}=45$; $Z=-2.978$; $P=0.002$; Fig. 3).

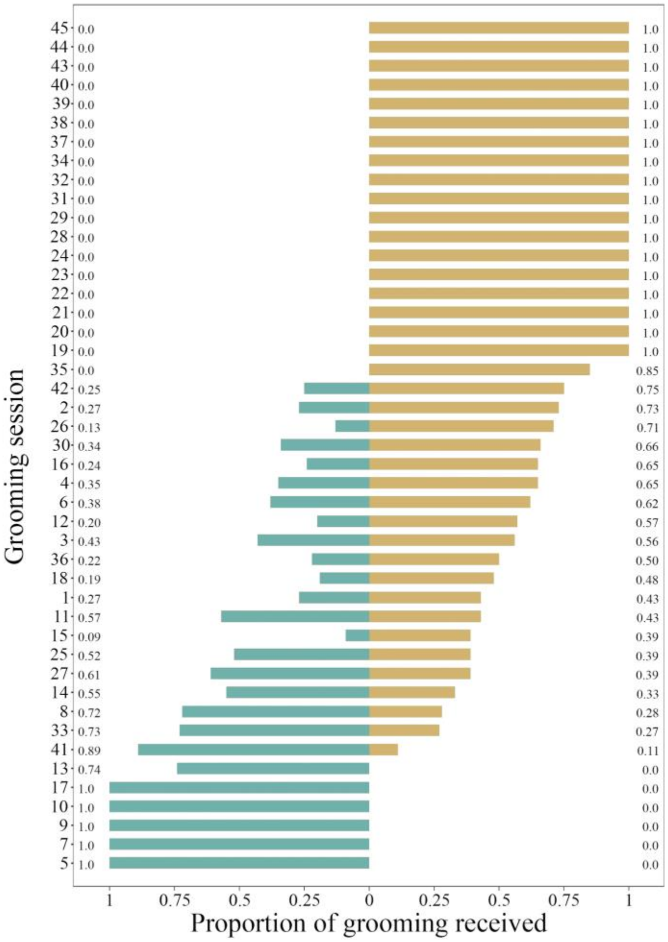

Figure 3 Bar plot showing that the proportion of grooming received in each grooming session by mothers (yellow bars) is higher than the proportion of grooming received by non-mothers (green bars) (Wilcoxon's test via Monte Carlo randomization: $N_{grooming\_sessions}=45$; $Z=-2.978$; $P=0.002$). The exact proportion of grooming received is shown on each line next to the reference bar

Prediction 1b – According to the LMM results (Table 3) the grooming sessions were longer in presence than in absence of infant handling (P=0.033; Fig. 2; effect size>1).

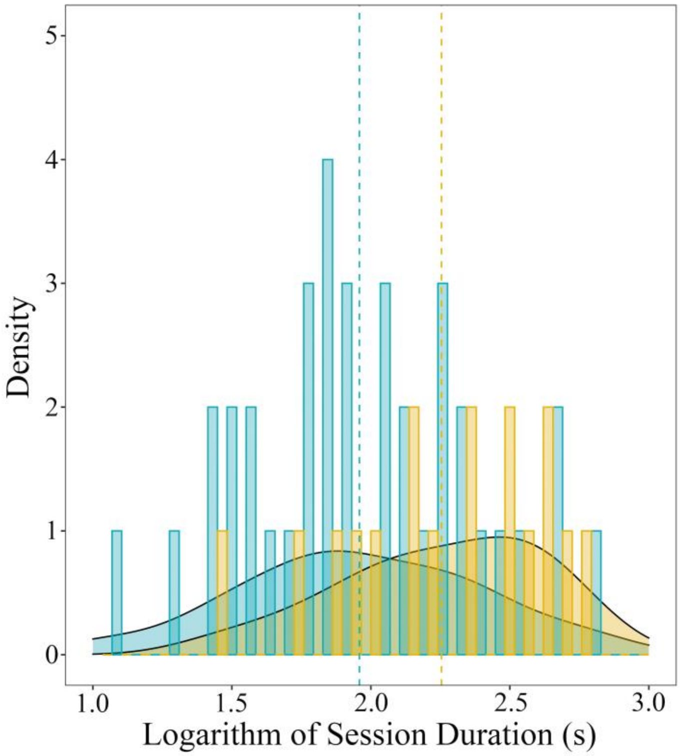

Figure 2 Barplot combined with a density plot showing that the duration of grooming sessions (log_{10} transformed data) is higher in presence (yellow bars) than in absence (blue bars) of infant handling (LMM: $N_{grooming\_sessions}$=55, P=0.021). The distribution of the duration of grooming sessions in presence and absence of infant handling is represented by the yellow and blue density curves, respectively. Dashed line indicates the mean grooming duration. Each bar indicates a grooming session.

Prediction 1c – The proportion of grooming received by the focal mother was higher in presence than in absence of infant handling (Wilcoxon Signed Rank test; $N_{focal\_mothers}$=8; Z=2.028; P=0.043; Fig. 4). In this analysis, we included only those focal mothers that had been involved in at least one grooming session with infant handling and one grooming session without infant handling.

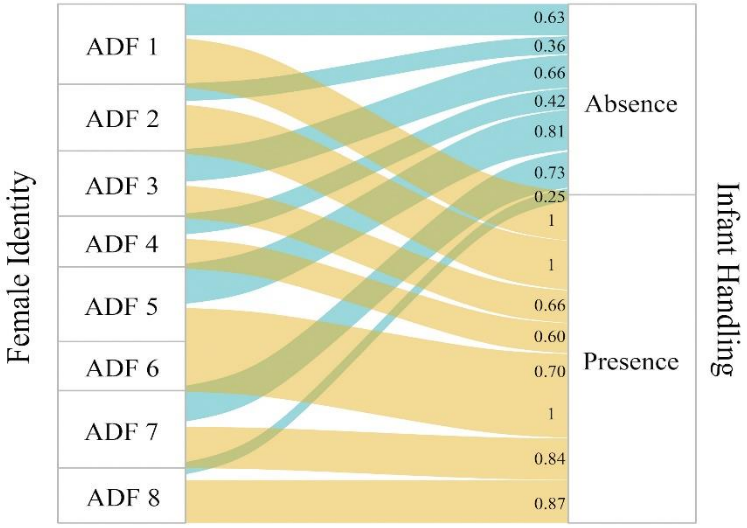

Figure 4 Alluvial plot (R package "ggalluvial") showing that the average proportion of grooming received by mothers is higher in presence (yellow bars) than absence of infant handling (blue bars) (Wilcoxon's test: $N_{focal\_mothers}$=8, Z=-2.028, P=0.043).

Prediction 2a - The correlation via randomization test did not reveal any significant effect of the number of in-group infants on the amount of grooming received by the mother within a single session (r = -0.103, $N_{grooming\_sessions}$=45; P=0.504).

Prediction 2b - Because there was a trend of significance in the effect of the infant age class on grooming duration (P=0.054, Table 3), we ran the Tukey test. The comparison between the three age classes did not show any significant difference (Tukey test; black infants *vs*. early infants, Est=0.097; SE=0.176; P=0.846; black infants *vs*. late infants, Est=0.274; SE=0.139; P=0.118; early infants *vs*. black infants, Est=0.177; SE=0.145; P=0.432).

Prediction 3

The behavioral sequence analysis carried out on the total grooming sessions ($N_{grooming\_sessions}=55$) showed significant transitions from the grooming given to the grooming received by the focal mother (grooming given → grooming received: P< 0.001), from grooming received to grooming given by the focal mother (grooming received → grooming given: P= 0.022), from infant handling to grooming received by the focal mother (infant handling → grooming received: P=0.006) and from grooming received by the focal mother to infant handling (grooming received → infant handling: P=0.004) (Fig. 5).

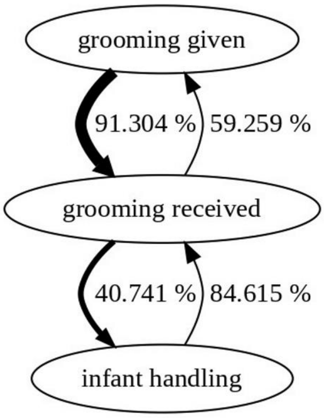

Figure 5 Flow diagram generated by Behatrix 0.9.11 representing the transitions and the percentage of occurrence between each behavior and the proceeding one. Thick arrows indicate significant transitions (grooming given → grooming received: P<0.001; grooming received → grooming given: P=0.022; infant handling → grooming received: P=0.006; grooming received → infant handling: P=0.004). 10 focal mothers were involved in 13 grooming session in which the grooming that they received followed infant handling.

Discussion

Our results show that infant handling in wild geladas is not only present but can also influence the grooming exchange between mothers and other females.

In line with prediction 1a, in our study groups grooming lasted longer when it involved a mother and a non-mother (Fig. 1, Table 3), with mothers receiving more grooming than non-mothers (Fig. 3) even though the grooming sessions between mothers (N=10) were fewer than the grooming sessions between mothers and non-mothers (N=45). This result - although preliminary - is in line with previous literature showing that primate mothers can be very attractive to the other females of the group (Hrdy, 1976; Maestripieri, 1994). Consistently, in different Old Word monkey species, non-mothers can groom mothers longer than other females even when infants become more independent (sooty mangabeys, *Cercocebus atys* and vervet monkeys, *Chlorocebus pygerythrus*: Fruteau et al., 2011; Tibetan macaques, *Macaca thibetana*: Jiang et al., 2019). Similarly, in moor macaques (*Macaca maurus*) Matsumura (1997) found that non-mothers groomed mothers significantly longer after than before birth, thus suggesting a possible linkage between grooming provided by the non-mothers and the presence of a newborn (Nicolson, 1987).

In contrast with prediction 1b, in our geladas the grooming sessions between females were longer in presence than in absence of infant handling (Fig. 2; Table 3). To our knowledge, Henzi and Barrett (2002) provided the only study addressing grooming duration in relation to the presence of infant handling and they found that in chacma baboons grooming sessions were shorter in presence of infant handling, possibly because non-mothers groomed mothers only the time that was necessary to obtain infant access. The divergence between chacma baboons (Henzi and Barrett, 2002) and geladas (present study) could be explained in the light of the different tolerance level of the two species. In chacma baboons, females show strict hierarchical relationships (Engh et al., 2006) and can be not so much tolerant with other females (Barrett et al., 2002). Thus, grooming can be necessary to obtain infant access. Geladas are characterized by high levels of tolerance and grooming exchange between females (Dunbar and Dunbar, 1975). In this respect, infant handling may further enhance female bonding via grooming, thus acting as a social bridge.

In agreement with prediction 1c, we found that mothers received more grooming when allowing their female grooming partners to manipulate their infants (Fig. 4). This result is in line with previous studies on other Old World monkey species. For example, in Tibetan macaques (Jiang et al., 2019), mothers allowing infant handling can receive a higher amount of grooming from other females compared to more protective mothers. Consistently, in olive baboons, Frank and Silk (2009) found that females preferentially groomed mothers, especially when infant handling was allowed. Similarly, in geladas the grooming exchange between mothers and non-mothers can be influenced by the presence of infant handling.

The number of available in-group infants did not correlate with the amount of grooming received by the mothers (prediction 2a not supported). Moreover, the infant age class did not affect the total duration of grooming sessions (prediction 2b not supported, Table 3). Thus, in geladas the exchange of infant handling and grooming does not seem to follow the supply/demand law. Two non-mutually exclusive explanations can account for this result. The first explanation relies upon the breeding period. In seasonally breeding primates, infants represent a variable and volatile commodity (Dunayer and Berman, 2016). For example, in vervet monkeys and sooty mangabeys (Henzi and Lucas, 1980; Mielke et al., 2020), Fruteau et al. (2011) found that mothers received less grooming by other females as the number of available infants and their age increased. In non-seasonal breeders such as geladas (Tinsley Johnson et al., 2018), black, early and late infants are available all year round, which results in very low fluctuations in the ratio between supply and demand. The second explanation could rely upon the tolerant nature of the species. In chacma baboons, characterized by low tolerance levels (Faraut et al., 2019), mothers are stressed by the attempts of others to access the infants and such stress increases when the number of infants is reduced (Henzi and Barrett, 2002). In this respect, non-mothers could provide a high amount of grooming to calm mothers and obtain infant access. In rather tolerant species (olive baboons: Frank and Silk, 2009; tufted capuchins monkeys: Tiddi et al., 2010) mothers are more relaxed and the number of infants does not appear to affect the amount of grooming provided by non-mothers, although the age of infants may have an effect depending on the species considered (Frank and Silk, 2009). In geladas, the high tolerance of females combined with the non-seasonal reproduction may explain why a biological market involving infants could not be necessary in geladas. Indeed, biological markets are unlikely to emerge in the presence of inter-individual relaxed relationships and when the level of permissiveness is high (Barrett et al., 1999). Further analyses on larger datasets are necessary to verify if infant age could influence the amount of grooming received by mothers and confirm that infants may not represent a 'commodity' in geladas.

Contrary to prediction 3, our sequential analysis revealed that infant handling either preceded or followed the grooming received by mothers (but not the grooming given by mothers) in a significant amount of transitions (Fig. 5). Thus, mothers could receive grooming regardless of whether the non-mothers had already handled the infant or not. A similar situation was found in patas monkeys (Muroyama, 1994) and olive baboons (Frank and Silk, 2009) where grooming could either precede or follow infant handling at comparable levels. In other case studies, a specific temporal association was found between the grooming received by mothers and infant-handling although with mixed results (Henzi and Barrett, 2002; Tiddi et al., 2010; Yu et al., 2013; Jiang et al., 2019). Specifically, in Tibetan macaques, the authors found that the grooming received by mothers followed infant handling (Jiang et al., 2019). Conversely, in chacma baboons (Henzi and Barrett, 2002), snub-nosed monkeys (Yu et al., 2013) and tufted capuchin monkeys (Tiddi et al., 2010) infant handling was found to precede the grooming received by mothers. Such differences in the time sequence between grooming received by mothers and infant handling may apply to the different social organizations, female-female bondings, and tolerance levels that characterize the different species. Geladas live in very tolerant, female bonded social groups and non-mothers do not need to provide grooming to mothers to access their infants. Instead, as discussed above, infant handling per se seems to enhance the grooming that females exchange and that mothers receive by non-mothers (regardless of whether it occurs before or after infant handling). Very few studies have so far addressed the temporal link between grooming and infant handling. Therefore, at this stage it is not possible to draw any convincing picture on what social features can produce the association between affiliation and infant access.

In conclusion, in geladas infant handling appears to be tolerated by mothers and works as a grooming enhancer. In this species, tolerance is so pronounced that the infants from different OMUs are left to play together by mothers (Dunbar and Dunbar, 1975; for the population of this study: Gallo et al., 2021). Although this study has to be considered as a preliminary investigation owing to the small sample size, our results show that in a tolerant species, such as geladas, infant handling may act as a "social bridge" that puts in contact mothers and non-mothers through grooming. In an evolutionary perspective, such a strategy might represent one of the stepping-stones to more complex forms of infant care, such as allomaternal care and cooperative breeding.

Ethical Statement

This study was approved by the Ethiopian Wildlife Conservation Authority (EWCA). Since the study was purely observational and was conducted with no manipulation of animals, no further permit was needed.

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CRediT authorship contribution statement

Marta Caselli: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Investigation, Methodology. **Anna Zanoli:** Data curation, Investigation, Methodology. **Elisabetta Palagi:** Conceptualization, Funding acquisition, Methodology, Supervision, Project administration, Writing – review & editing. **Ivan Norscia:** Conceptualization, Funding acquisition, Methodology, Supervision, Project administration, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Supplementary material

Video S1

The video shows an example of a grooming session from a non-mother to a mother, followed by infant handling. Video by Ivan Norscia (Panasonic Lumix DMC-FZ60)

https://www.sciencedirect.com/science/article/pii/S0376635721001856?casa_token=gi0_V7UA-3cAAAAA:lMG81fkz1e35vIGzCLrz-VrtZCwq0Jkn3Xx1uMuuNM9zG4zBQqRZ8xk1Xy7FhZ2WiqlGByQnhg

CHAPTER 3

The face never lies: facial expressions and mimicry modulate playful interactions in wild geladas

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The face never lies: facial expressions and mimicry modulate playful interactions in wild geladas

Alessandro Gallo[1,2], Anna Zanoli[1], Marta Caselli[1], Ivan Norscia* [1, 3], Elisabetta Palagi* [3, 4]

[1] University of Turin, Department of Life Sciences and Systems Biology, Via Accademia Albertina 13, 10123 Torino (Italy)

[2] University of Rennes, Normandie University, CNRS, EthoS (Éthologie animale et humaine) – UMR 6552, 35000, Rennes (France)

[3] Natural History Museum, University of Pisa, Via Roma 79, 56011 Calci (Pisa, Italy)

[4] Unit of Ethology, Department of Biology, University of Pisa, Via A. Volta 6, 56126 Pisa (Italy)

* Elisabetta Palagi and Ivan Norscia share the senior authorship

Corresponding authors: elisabetta.palagi@unipi.it; ivan.norscia@unito.it

Elisabetta Palagi ORCID: 0000-0002-2038-4596

Ivan Norscia ORCID: 0000-0002-1618-7717

Abstract

Play fighting, the most common form of social play in mammals, is a fertile field to investigate the use of visual signals in animals' communication systems. Visual signals can be exclusively emitted during play (e.g., play faces, PF, context-dependent signals), or they can be released under several behavioral domains (e.g., lip-smacking, LS, context-independent signals). Rapid facial mimicry (RFM) is the involuntary rapid facial congruent response produced after perceiving others' facial expressions. RFM leads to behavioral and emotional synchronization that often translates into the most balanced and longest playful interactions. Here, we investigate the role of playful communicative signals in geladas (*Theropithecus gelada*). We analyzed the role of PF and LS produced by wild immature geladas during play fighting. We found that PFs, but not LS, were particularly frequent during the riskiest interactions such as those including individuals from different groups. Furthermore, we found that RFM (PF→PF) was highest when playful offensive patterns were not biased towards one of the players and when the session was punctuated by LS. Under this perspective, the presence of context-independent signals such as LS may be useful in creating an affiliative mood that enhances communication and facilitates most cooperative interactions. Indeed, we found that sessions punctuated by the highest frequency of RFM and LS were also the longest ones. Whether the complementary use of PF and LS is strategically

guided by the audience or is the result of the emotional arousal experienced by players remains to be investigated.

Significance Statement

Facial expressions and their rapid replication by an observer are fundamental communicative tools during social contacts in human and non-human animals. Play fighting is one of the most complex forms of social interactions that can easily lead to misunderstanding if not modulated through an accurate use of social signals. Wild immature geladas are able to manage their play sessions thus limiting the risk of aggressive escalation. While playing with unfamiliar subjects belonging to other groups, they make use of a high number of play faces. Moreover, geladas frequently replicate others' play faces and emit facial expressions of positive intent (i.e., lip-smacking) when engaging in well-balanced long play sessions. In this perspective, this "playful facial chattering" creates an affiliative mood that enhances communication and facilitates most cooperative interactions.

Keywords: Play faces, Lip-smacking, Play fighting, Play units, Visual communication, *Theropithecus gelada*

Introduction

Communication is the process during which senders produce specific designed displays to modify receivers' behaviors (Hebets and Papaj, 2005). Due to its variability and unpredictability, social play is a good domain to test hypotheses on the evolution of signals that can have the immediate function of fine-tuning the session (Palagi et al., 2016). The most frequent type of social play is play fighting which includes motor actions often recruited from the agonistic context (Pellis et al., 2010a, b; Pellis and Burghardt, 2017; Pellis and Pellis, 2017; Nolfo et al., 2021). This type of play represents a challenge for animals that need to rapidly adopt flexible tactics and adjust their actions according to the intrinsic characteristics of the playmates (i.e., age, sex, kin) and the contexts under which the session is taking place (Pellegrini, 2009). To be successful, play fighting requires sophisticated skills in performing actions and anticipating those of others (Fagen, 1993; Spinka et al., 2001). This fine adjustment appears to be even more important when play fighting involves players of different ages, sexes, sizes and groups. A play fighting session involves actions that are borrowed from other functional contexts such as real aggression, submission and mother-infant behaviors. Playful patterns can therefore be defined as advantageous or disadvantageous as a function of the different domains in which they originate (Burghardt, 2005; Pellis and Pellis, 2009). To maintain a playful mood, individuals can alternate their advantageous/disadvantageous actions to make the session less ambiguous and well balanced (Gallo et al., 2021). In this respect, when a player gives the playmate the possibility to counterattack (e.g., role-reversal) or limits its strength by finely controlling motor actions (e.g., with self-handicapping behaviors); this actively contributes to make the session more balanced (symmetric) and prolonged (Pellis and Pellis, 2009). However, role-reversal and self- handicapping per se are not always effective to modulate the session and so signals may be needed for the session to be maintained (de Waal, 2003; Palagi, 2008; Pellis and Pellis, 2009).

Play communication, which often relies on multiple sensory systems, can be either unimodal or multimodal (Winkler and Bryant, 2021). Since play fighting requires a strong physical closeness between the players, visual signals acquire particular importance in this domain (Palagi, 2008, 2009). Body postures, gestures and facial expressions often punctuate the play sessions in several animal species (Fagen, 1981; Bekoff, 2001; Palagi, 2006; Palagi et al., 2016). Such visual signals can be specific to the play domain (context-dependent signals) or can convey different meanings depending on the species and context in which they are displayed (context-independent signals) (Maestripieri, 1997; Palagi and Mancini, 2011).

One of the typical context-dependent signals is the relaxed open mouth or play face (van Hooff and Preuschoft, 2003; Davila-Ross and Dezecache, 2021), a facial expression present in several primate and non-primate species (e.g., American black bear, *Ursus americanus*, Henry and Herrero, 1974; domestic dogs, *Canis lupus familiaris*, Palagi et al., 2015; South American sea lions, *Otaria flavescens*, Llamazares-Martín et al., 2017a; ring-tailed lemurs, *Lemur catta*, Palagi et al., 2014; Norscia and Palagi, 2016; Barbary macaques, *Macaca sylvanus*, Preuschoft, 1992; lowland gorillas, *Gorilla gorilla gorilla*, Palagi et al., 2007; chimpanzees, *Pan troglodytes*, Palagi, 2006; bonobos, *Pan paniscus*, Palagi, 2008). The play face is used to express positive emotions and anticipate the affiliative nature of the behaviors that are going to be enacted (i.e., metacommunicative signal) (de Waal, 2003; Pellis and Pellis, 2009; Demuru et al., 2015). Among the facial displays expressing positive intentions, lip-smacking is one of the most versatile in primates because it has been found in several contexts such as infant caring, subordination and affiliation, according to the different level of tolerance of a species (van Hooff, 1962; Thierry, 1984; Preuschoft, 1992, 1995; Scopa and Palagi, 2016). For example, in macaque species, lip-smacking, along with the silent-bared-teeth display, can be expressed in different contexts and conveys different meanings. In Japanese macaques, a highly despotic species, the silent-bared-teeth and lip-smacking are emitted by subordinates to signal their low-ranking status (de Waal and Luttrell, 1985; Maestripieri, 1996; Preuschoft, 1995; Shimooka and Nakagawa, 2014). In Tonkean macaques, a highly tolerant species, lip-smackingis present both during affiliative (Thierry, 1984; Preuschoft, 1995; Micheletta et al., 2013) and playful contacts (Pellis et al., 2011; Scopa and Palagi, 2016) to convey a message of positive mood.

Whatever the kind of signal emitted, to make the exchange of information effective, both players need to be attentive to the face of the other so that they can correctly decode the signal and respond appropriately (Palagi and Mancini 2011). The receiver can reply with a simple playful pattern or by replicating the exact facial expression of the sender in a rapid (<1 s), unconscious way, a phenomenon known as rapid facial mimicry (RFM, Fig. 1a). The phenomenon of RFM has been demonstrated in several non-primate (*Canis lupus familiaris,* Palagi et al., 2015; *Suricata suricatta*, Palagi et al., 2019a; *Helarctos malayanus*, Taylor et al., 2019) and primate species (*Theropithecus gelada*, Mancini et al., 2013a; *Pongo pygmaeus*, Davila-Ross et al., 2008; *Pan troglodytes* and *Gorilla gorilla gorilla*, Palagi et al., 2019b). RFM can involve the perception-action mechanism and can have a role in emotion recognition (Palagi et al., 2020; Nieuwburg et al., 2021). By its potential role in favoring emotional sharing, RFM can have implications in determining the success of the play sessions. It has been demonstrated that the occurrence of RFM during play encounters fosters motor synchronization that translates into longer playful interactions (Mancini et al., 2013b; Palagi et al., 2015, 2019a; Scopa and Palagi, 2016).

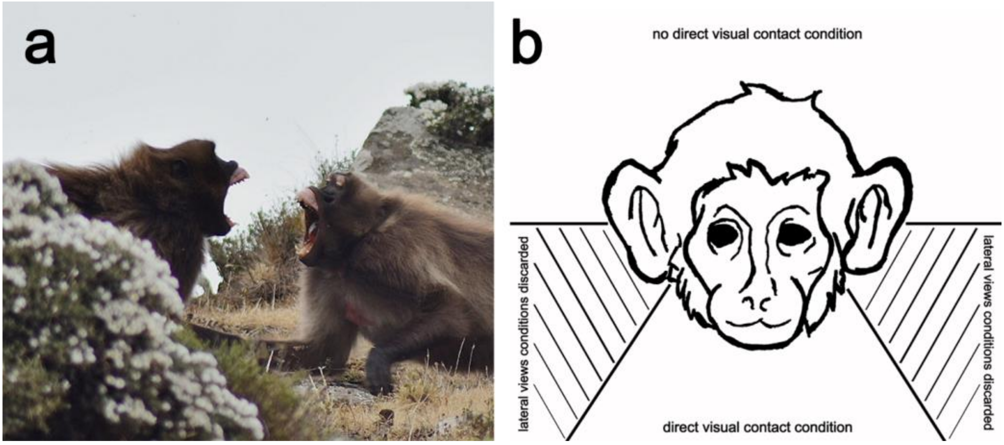

Figure 1 a Picture showing two subadult playmates displaying Rapid Facial Mimicry (RFM) of the play face (Photo credits Alessandro Gallo). **b** Drawing showing the possible visual conditions during the expression of play faces. For the analyses we considered a play face as perceived if subjects were in the 'direct visual contact condition', and as not perceived if subjects were in the 'no direct visual contact condition'. The doubtful cases falling in the 'lateral views conditions' were discarded from the analyses

A good species to test hypotheses on playful communication is *Theropithecus gelada*. The gelada is a monkey species endemic to Ethiopia organized in a multilevel social system (Dunbar and Dunbar, 1975) whose basic module is the one-male unit (OMU) composed of one leading male, several females and their offspring (Snyder-Mackler et al., 2012). Furthermore, inter-unit relationships are not based on social exchange and affiliation but on a high level of tolerance among different OMUs (Snyder-Mackler et al., 2012). The specific spatial aggregation maintained by the units underlines the extraordinary level of inter individual tolerance characterizing geladas' complex fluid system (le Roux et al., 2011).

Since geladas mostly rely on grass for foraging, it has been supposed that their feeding ecology is at the basis of the high levels of tolerance between groups. The impossibility of monopolizing such an abundant resource leads to low levels of food competition not only at inter- but also at intra-unit level (le Roux et al., 2011). Within the OMU, geladas are characterized by relationships not strictly codified by rigid rank rules (Bergman, 2010; Palagi and Bergman, 2021) and, compared to other *Cercopithecine* species, group integrity is maintained by the strong affiliation among the individuals more than their dominance interactions (le Roux et al., 2011; Snyder-Mackler et al., 2012).

Different OMUs spend most of their time in proximity thus favoring the formation of fluid groups of infants and juveniles belonging to both sexes (i.e. play units) that engage in high levels of playful contacts (wild: Dunbar and Dunbar, 1975; Gallo et al., 2021; captive: Palagi and Mancini, 2009, 2011). Hence, play is the only positive interaction linking subjects belonging to different OMUs (Dunbar and Dunbar, 1975). Such playful encounters can vary in their competitive elements according to the level of familiarity of players (Gallo et al., 2021). Among primates, geladas possess one of the richest repertoires of facial expressions (Dunbar and Dunbar, 1975; Leone et al., 2014; Lazow and Bergman, 2020; Zanoli et al., 2021) that are performed in a highly flexible way under different contexts. As it occurs in Tonkean macaques, also geladas lip-smacking has a role in modulating affiliative interactions (e.g., reconciliation: Leone and

Palagi, 2010; consolation: Palagi et al., 2018) and can be performed during playful interactions (Palagi and Mancini, 2011). Finally, geladas are the first monkey species in which the phenomenon of RFM has been demonstrated (Mancini et al., 2013a).

Here, we investigate how wild geladas adapt their playful communicative signals by testing the following predictions.

Prediction 1: occurrence of play signals - The asymmetry of a session can be measured by the play asymmetry index (PAI) that takes into account the number of advantageous, disadvantageous and neutral patterns each player engages in. The highest scores of PAI correspond to highly unbalanced session (see “Methods” for the mathematical formula). In wild geladas, play fighting is less symmetric and cooperative (more unbalanced) between subjects belonging to different OMUs than between individuals belonging to the same OMU (Gallo et al., 2021). The unbalanced nature of inter-OMU play translates into sessions characterized by a high degree of instability and short duration (Gallo et al., 2021). To avoid the risk of misunderstanding and escalation into real aggression, play fighting requires a clear use of signals (Aldis, 1975; Palagi, 2008, 2009). If play face (PF) and lip-smacking (LS) are signals that convey messages of positive intent (Maestripieri and Wallen, 1997; de Waal, 2003; Pellis and Pellis, 2009), we expect to find these facial displays (PF, Prediction 1a; LS, Prediction 1b) to be more frequent during inter-OMU than during intra-OMU play sessions, as the former are characterized by the highest levels of asymmetry (see Gallo et al., 2021).

Prediction 2: frequency and effects of RFM on the play sessions - In geladas, RFM is present for context-dependent (e.g., play faces) but not for context-independent signals (e.g., lip-smacking) (Mancini et al., 2013a); hence, hereafter, we will refer to RFM in relation to play faces. This phenomenon improves the exchange of communicative signals between players thus leading to prolonged sessions (Mancini et al., 2013b). Little is known about the possible factors influencing RFM in geladas, although social bonding (e.g., kinship) seems to positively modulate and reinforce the phenomenon (Mancini et al., 2013a). If RFM is positively predicted by the level of familiarity shared by players, we predict facial mimicry to be more frequent when the session involves subjects of the same group (intra-OMU play) than when it involves subjects of different groups (inter-OMU play) (Prediction 2a). If RFM is predicted by playful synchronization and mood sharing between subjects(Mancini et al., 2013b; Palagi et al., 2015, 2019a; Scopa and Palagi, 2016), we expect the phenomenon to increase when the players engage in more balanced interactions characterized by low PAI values (Prediction 2b). Since the most balanced sessions are also the longest ones (Waller and Dunbar, 2005; Davila-Ross et al., 2008, 2011; Gallo et al., 2021), if RFM has a role in balancing the session by fostering motor synchronization, we predict a positive correlation between the number of RFM events and the duration of the session (Prediction 2c).

Methods

The species and data collection

The study was conducted on a population of wild geladas living on the Kundi plateau (Wof-Washa area, Amhara region, Ethiopia, N9°40.402′ E39°45.060′) from January to May 2019 (during both dry and early wet seasons). Two pairs of observers collected data by video recordings (Pana-sonic HC-V180 Full HD optic-zoom 50×, 2 s accuracy) and monitored two different parts of the plateau (Northern and Southern part, each part about 0.1 km^2). For this study, we used data from 14 one-male units (OMUs) out of the 21 groups composing the total population of geladas living on the Kundi plateau (Caselli et al., 2021; Gallo et al., 2021).

The observers registered the OMUs' identity and the number of subjects present in a range of 50 meters via instantaneous scan sampling (10-min intervals, Altmann, 1974). After recognizing the OMUs present in such a range, the observers video-recorded the activities between immature subjects (video length from 5 to 20 min). While the first observer managed the camera, the second one described the ongoing activities (e.g., play activities, social interactions, proximity between players and their mothers), the identity of the subjects and their group membership. Therefore, the videos also included audio descriptions of the scenes and the subjects involved. When it was not immediately possible to determine the group membership of the players, the observers prolonged the registration until the OMUs split into single ones, thus permitting an a posteriori assignment of the players to their exact OMUs. We collected about 120h of video, including 2 h of playful interactions ($N_{session}$=527). It was not possible to record data blind because our study involved focal animals in the field.

Video analysis and operational definitions

By using the software VLC, we performed a video analysis on the 527 playful sessions recorded. To collect all the information required for an accurate analysis, we analyzed each video-recorded playful session frame-by-frame (200-s accuracy). The video analysis was conducted by two observers and started after a training period conducted by EP and when the Cohen's Kappa between the observers was never below 0.75.

A dyadic playful session started with an approach and a playful pattern directed by a subject towards a groupmate. The session ended when one of the two players moved away. Two sessions were considered different if divided by an interval of at least 10 s (Mancini and Palagi, 2009; Gallo et al., 2021).

For each playful pattern of a given play session, we recorded the OMU membership of the players (intra-OMU *vs*. inter-OMU), players' age- and sex-class following the criteria used in Dunbar and Dunbar (1975) and Gallo et al. (2021).

We also recorded the duration (in seconds), the exact sequence of the patterns displayed, play faces (inter-observer reliability of the two observers, Cohen's Kappa 0.89), lip-smacking (Cohen's Kappa 0.84) and RFM events (Cohen's Kappa 0.96). When it was not possible to determine the sex of either player ($N_{session}$=49), the sex-class was assigned randomly (as per Dunbar and Dunbar, 1975). We included in the statistical analysis the dyadic playful interactions that were composed of at least three motor patterns and during which the players were always visible ($N_{session}$=252). Since seasonality can affect the frequency of animal play (Barrett et al., 1992; Fagen, 1993), following the season's classification included in Caselli et al. (2021), we divided our playful sessions according to the season in which they occurred (wet season: from January to March; dry season: from April to May).

Play Asymmetry Index (PAI)- PAI was calculated based on advantageous (ADV), disadvantageous (DIS) and neutral play patterns (NEUTRAL) (Bauer and Smuts, 2007; Ward et al., 2008; Cordoni et al., 2016; Llamazares-Martín et al., 2017b; Palagi et al., 2019a; Nolfo et al., 2021). A pattern was considered as ADV when a player directed an offensive pattern or when it was in a favorable position with respect to the playmate (e.g., an animal bites the other; an animal recovers a thing from the playmate). Therefore, the ADV category does not exclusively include offensive patterns. A pattern was scored as DIS when a player assumed a defensive or a self- handicapping position (e.g., head rotation, play crouching). So, if a gelada engaged in an ADV pattern (e.g., biting) and the playmate counterattacked with another ADV pattern (e.g., biting), the outcome of the interaction would be balanced. The same occurs for the DIS patterns. All these patterns can occur outside or during a rough and tumble sequence. When it was not possible to clearly categorize ADV or DIS patterns within a rough and tumble interactions, the short sequence of rough and tumble was categorized as neutral. A neutral pattern is an action that does not provide any ADV or DIS to the player. See Table S1 for the categorization of the playful patterns as advantageous, disadvantageous and neutral. The formula for the PAI calculation is the following:

$$\frac{(ADV\ A + DIS\ B) - (ADV\ B + DIS\ A)}{(ADV\ A + DIS\ B) + (ADV\ B + DIS\ A) + NEUTRAL}$$

The PAI was calculated for each dyadic playful session. To measure the overall asymmetry of each session, we used the absolute PAI value (|PAI|), which ranges from 0 (perfectly balanced session) to 1 (completely unbalanced session).

Rapid facial mimicry (RFM)

To examine the presence of RFM, defined as matching response produced by the receiver within 1 s after the perception of the triggering stimulus (Mancini et al., 2013a), we scored both play faces and full play faces as unique categories (PF) since they represent the two configurations of the relaxed open-mouth display (Palagi, 2018). In this way, when we calculated the RFM, we did not consider the exactmatching response (PF/PF; FPF/FPF) but, more generally, the congruent response (PF/PF; FPF/FPF; PF/FPF; FPF/PF) by following the previous results available for this species (Mancini et al., 2013a).

We focused on the face of one individual (the observer) to verify whether, within 1 s, it varied as a function of the facial expression performed by the sender (hereafter, the trigger). The trigger was defined as the first playmate that emitted the stimulus (PF).

To reliably evaluate that the trigger's PF was actually the eliciting stimulus of the observer's PF, we considered only those events in which the observer, while looking at the trigger's face, did not perform any PF in the second before the perception of the trigger's PF.

To evaluate the possibility for the observer to perceive the PF emitted by the trigger, we considered the observer's head orientation (Demuru et al., 2015; Scopa and Palagi, 2016). We considered the PF as perceived when the trigger was in direct visual contact with the observer (i.e., within the range of its stereoscopic view). When the observer was facing away from the trigger (no direct visual contact), we considered the PF as not perceived. All the uncertain cases due to lateral views were discarded (see Fig. 1b). The Cohen's Kappa for direct and no-direct visual contacts was 0.88.

Under the perception condition, the latency of the mimicry response was measured as follows: from the first frame corresponding to the first trigger's lip opening to the first frame corresponding to the first observer's lip opening.

Statistics

The statistical analyses were conducted in R (version 3.5.3, R Core Team, 2019). To investigate if PFs were most frequently displayed during inter-OMU play (prediction 1a), we ran a linear mixed model ($Model_1$; $N_{observations}=252$) with the function *lmer* of the R-package 'lme4' (Bates et al., 2015). We verified the normal distribution and homogeneity of the residuals by looking at the qqplot and plotting the residuals against the fitted values (Estienne et al., 2017). Due to the non-normal distribution of the residuals, the frequency of PF (outcome variable = number of PF/play session duration) was transformed in a logarithmic scale. For this model, the fixed factors were as follows: OMU membership (Inter-OMU, Intra-OMU), sex of the players (same-sex, different-sex), age of the players (same-age, different-age), |PAI|, lip-smacking (presence, absence), season (wet, dry). To investigate the distribution of LS in inter- and intra-OMU play sessions (Prediction 1b), we ran a generalized linear mixed model ($Model_2$; R-package 'glmmTMB', Brooks et al., 2017) with LS frequency (number of LS/play session duration) as the outcome variable ($N_{observations}=252$). Since the residuals were non-normally distributed, even when log-transformed, we chose a Beta distribution after fitting the model using the R-*fitdist* function (Delignette-Muller and Dutang, 2015). For this model, the fixed factors were both used in the PF model, excluding the presence/absence of LS. To verify the occurrence of RFM events, we used a reduced dataset composed only of those sessions in which there were at least two play faces for both the players involved ($N_{observations}$=116).

To evaluate whether OMU membership (Prediction 2a) and PAI values (Prediction 2b) influenced the frequency of RFM, we ran a third linear mixed model ($Model_3$; Bates et al., 2015) for which the outcome variable was the ratio between the total number of RFM events and the frequency of PF in the logarithmic scale (to reach a normal distribution of the residuals). We used the same fixed variables as the $Model_1$. In all the models, the playing dyad was entered as a random factor. We verified if the full models (including all the fixed factors) significantly differed from the null models (including only the random factor) by using a likelihood ratio test (ANOVA with argument 'Chisq'; Dobson, 2002).

Finally, to test if the frequency of RFM predicted the duration of the play sessions ($N_{observations}$=116) (prediction 2c), we ran a fourth model ($Model_4$: Bates et al., 2015). We built the model using the logarithm of the session's duration (sec) as the outcome variable (after checking the normal distribution of residuals with the same method used for the PF model). The fixed factors were LS (presence, absence) and the total number of RFM events/frequency of PF. The influence on play duration of the OMU membership (inter-OMU, intra-OMU), sex of the players (same-sex, different-sex), age of the players (same-age, different-age), |PAI| and season (wet, dry) has already been tested in Gallo et al. (2021). For this reason, these variables were included in the LMM as control predictors. The playing dyad was considered as a random factor. To test the significance of the full model (Forstmeier and Schielzeth, 2011), we compared it against a null model which comprised the control predictors and the random factor only, by using a likelihood ratio test (ANOVA with argument test 'Chisq'; Dobson, 2002). For all four models, we checked variance inflation factors (VIF) using the 'car' package (Fox and Weisberg, 2011) and we calculated the *p*-values of each predictor based on likelihood ratio tests between the full and the null models (R-function *drop1*, Barr et al., 2013).

Results

Frequencies of play faces (PF) and lip-smacking (LS) in intra- and inter-OMU play (Model$_1$; Model$_2$)

When investigating which variables predicted the frequency of playful facial expressions (PF model, Model$_1$), we found that the full model significantly differed from the null model (χ^2=14.405, df=6, P=0.025; Table 1a). No collinearity was found between the predictors (VIF$_{min}$=1.007; VIF$_{max}$=1.106). The frequency of PFs was significantly predicted by OMU membership, with the playmates belonging to different OMUs performing the highest level of PFs (Table 1a; Fig.

2) (prediction 1a supported). Focusing on the variables possibly affecting the frequency of lip-smacking (LS model, $Model_2$), we found that the full model did not significantly differ from the null model (χ^2=1.915, *df*=8, P=0.860) (prediction 1b not supported).

Table 1 Results of the LMMs showing the effects of the fixed factors on play face frequency (a), RFM frequency (b) and duration of the session (c)

a – Play face frequency (full *vs* control model: χ^2=14.405, *df*=6, P=0.025)					
Fixed factors	**Estimate**	**SE**	***Df***	**Z**	**P**
Intercept	-0.304	0.088	A	-3.473	a
Sex (Mismatched)[b,c]	-0.017	0.050	252	-0.344	0.731
Age (Mismatched)[b,c]	-0.026	0.043	252	-0.599	0.550
OMU (Intra)[b,c]	-0.122	0.043	252	-2.836	**0.005**
absPAI	0.073	0.075	252	0.970	0.333
Lipsmack (Presence)[b,c]	-0.083	0.071	252	-1.172	0.242
Season (Wet)[b,c]	-0.142	0.078	252	-1.812	0.071
b - RFM frequency (full *vs* control model: χ^2=25.750, *df*=6, P=0.00025)					
Fixed factors	**Estimate**	**SE**	***Df***	**Z**	**P**
Intercept	0.643	0.105	a	6.098	a
Sex (Mismatched)[b,c]	-0.013	0.072	116	-0.183	0.855
Age (Mismatched)[b,c]	-0.003	0.060	116	-0.049	0.961

OMU (Intra)[b,c]	0.098	0.059	116	1.664	0.099
absPAI	-0.405	0.111	116	-3.661	**0.000**
Lipsmack (Presence)[b,c]	0.208	0.083	116	2.510	**0.013**
Season (Wet)[b,c]	0.156	0.096	116	1.638	0.104

c – Duration of the session (full *vs* control model: χ^2=91.076, *df*=2, P< 0.001)

Fixed factors	**Estimate**	**SE**	***df***	**Z**	**P**
Intercept	0.926	0.077	a	12.036	a
Sex (Mismatched)[b,c]	0.103	0.052	109.683	1.998	0.048
Age (Mismatched)[b,c]	0.009	0.043	110.139	0.208	0.835
OMU (Intra)[b,c]	0.102	0.043	108.186	2.395	0.018
absPAI	-0.238	0.078	107.787	-3.032	0.003
Lipsmack (Presence)[b,c]	0.123	0.058	90.398	2.140	**0.035**
Season (Wet)[b,c]	0.050	0.068	112.682	0.734	0.464
RFM frequency	0.036	0.003	105.289	10.998	**0.000**

[a] Not shown as not having a meaningful interpretation
[b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor
[c] These predictors were dummy coded, with the "Sex (Matched)", "Age (Matched)", "OMU (Inter)", "Lipsmack (Absence)", "Season (Dry)" being the reference categories

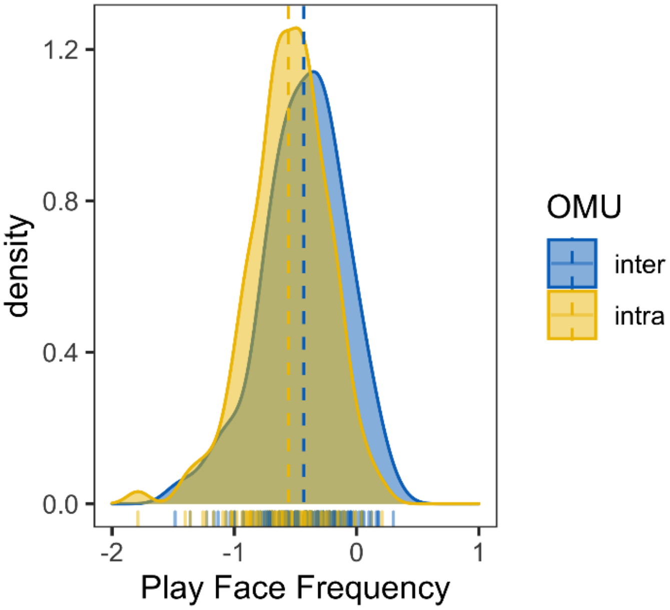

Figure 2 Density plot, drawn with the R-package 'ggpubr' (Kassambara, 2020), showing the frequency of Play Faces in the inter-OMU (blue density curve) and in the intra-OMU (yellow density curve) conditions. Individual observations are presented under the density curves with pipe symbols. Dotted lines represent the mean values for each condition.

Role of familiarity and play asymmetry on the frequency of RFM (Model$_3$)

The full model built to evaluate which variables predicted the distribution of RFM significantly differed from the null model (χ^2=25.750, *df*=6, P< 0.001; Table 1b). No collinearity was found between the fixed factors (VIF$_{min}$=1.009; VIF$_{max}$=1.108). The frequency of RFM was positively predicted by the low levels of |PAI| (Fig. 3a; Table 1b; prediction 2b supported). Moreover, the presence of LS was associated with the highest frequency of RFM (Fig.3b). The level of familiarity (OMU membership) did not influence the distribution of RFM during the playful session (Prediction 2a not supported).

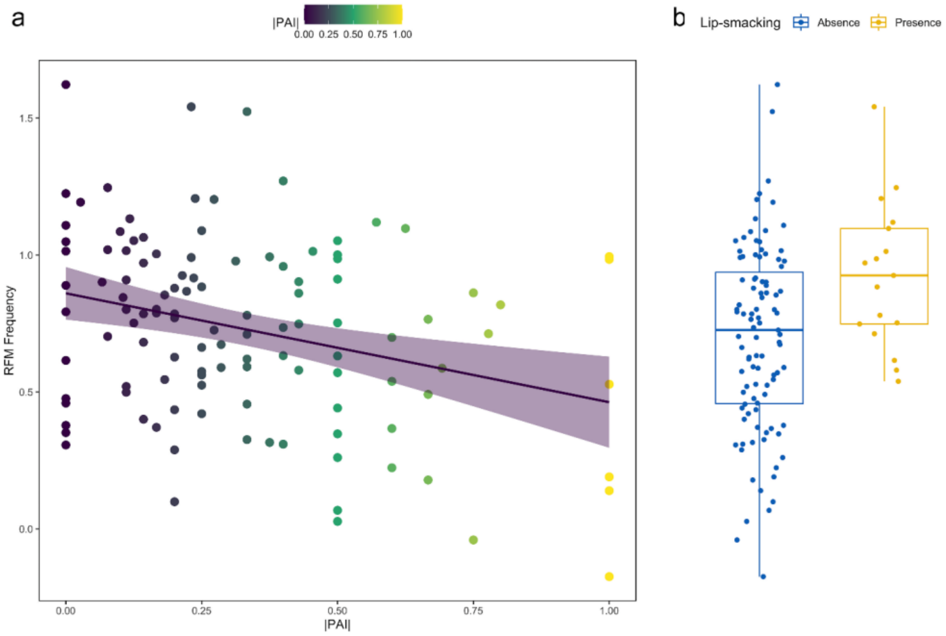

Figure 3 a Scatter plot showing the relationship between the frequency of RFM and the Play Asymmetry Index absolute value (|PAI|). Dots' color follows the variation of |PAI| values. The purple line represents the linear regression between the variables and the respective confidence interval. **b** Box plot showing the RFM frequency variation in absence (blue box) and in presence (yellow box) of Lip-smacking. (Plots were created in R using the 'ggplot2' package; Wickham, 2016). Boxes indicate the inter-quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR

Effect of RFM and LS on play duration (Model$_4$)

Finally, when testing if the frequency of RFM and the presence of LS predicted the duration of the session, the full model significantly differed from the null model (χ^2=91.076, *df*=2, P< 0.001; Table 1c). No collinearity was found between the predictors (VIF_{min}=1.024; VIF_{max}=1.124). The frequency of RFM (Fig.4a) was positively associated with the duration of the playful sessions (prediction 2c supported). Moreover, the longest sessions were characterized by the presence of LS (Fig.4b).

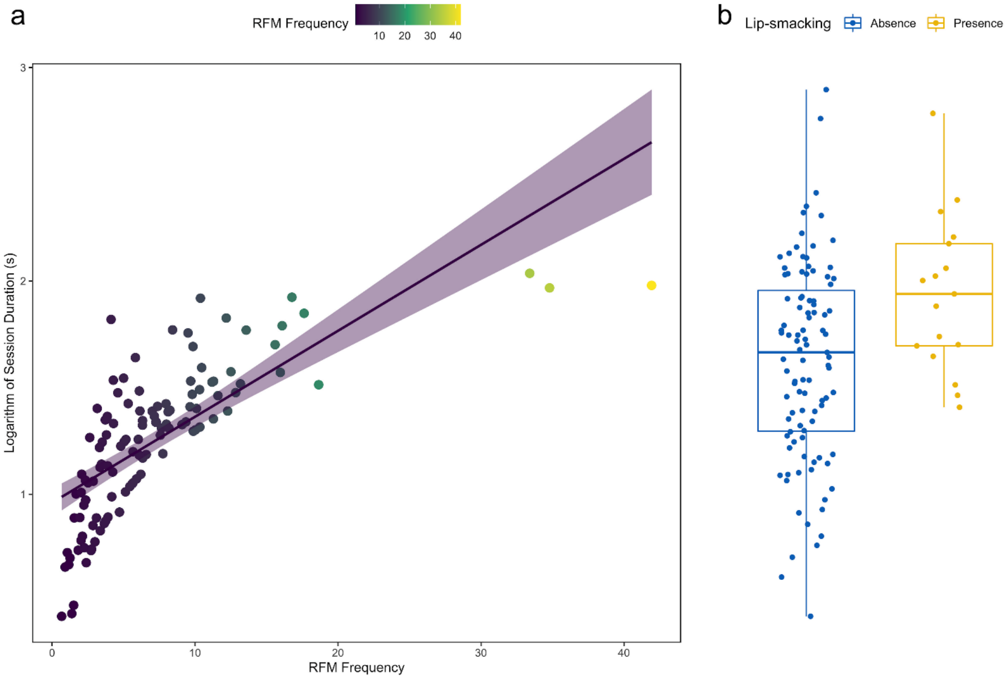

Figure 6 a Scatter plot showing the relationship between the logarithm of the play session duration and the frequency of RFM. Dots' color follows the variation of RFM frequency. The purple line represents the linear regression between the variables and the respective confidence interval. **b** Box plot showing the variation of the play session duration in absence (blue box) and in presence (yellow box) of Lip-smacking. (Plots were created in R using the 'ggplot2' package; Wickham, 2016). Boxes indicate the inter-quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR

Discussion

Here, we focused on the role of visual communication signals in the playful domain in a wild monkey species, the gelada. Our findings show that context-dependent facial expressions (play faces) have a role in the management of playful interactions (prediction 1a supported). In wild geladas, contrary to lip-smacking (predicting affiliation in tolerant species, Thierry, 1984, 1985, 1986; Preuschoft, 1992, 1995; Matsumura, 1994, 1997; Maestripieri and Wallen, 1997; Palagi and Mancini, 2011), the use of play faces depended on playmates' group membership (Table 1a). The incidence of RFM (Fig. 1a), a phenomenon demonstrated only for the play faces in this species (Mancini et al. 2013a), was predicted by the playful tactics (i.e., most cooperative play) (Table 1b; Fig. 3b; prediction 2b supported) and the occurrence of lip-smacking (Table 1b; Fig. 3a).

Gallo et al. (2021) demonstrated that inter-OMU play fighting is more competitive (i.e., most asymmetric play sessions) than intra-OMU play fighting. In the present study, play faces were particularly frequent when the players did not belong to the same OMU (Fig.2) thus suggesting that unfamiliar subjects, when facing such competitive interactions, need signal redundancy to communicate the playful nature of their agonistic patterns (Prediction 1a supported). Our findings on wild geladas are in line with data coming from captive populations. Palagi and Mancini (2011) found that when the session involved age-mismatched dyads (e.g., adult-infant), the adult subjects engaged in the highest rates of play faces. Similar evidence also comes from primate species showing high levels of despotism such as ring-tailed lemurs (Pereira and Kappeler, 1997). In this species, the use of play faces is redundant when play fighting becomes highly unbalanced independently from the age-matching of the players (Palagi et al., 2014). Signal redundancy is also present in chimpanzees that can strategically modulate their play faces during high-risk situations. When playing with infants, adolescent chimpanzees can enhance the use of play faces when they are observed by the mothers of their playmates to possibly adjust their facial displays depending on a specific audience when the play became rough (Flack et al., 2004). An additional example on the importance of play faces during sessions involving a certain degree of risk comes from bonobos (Palagi, 2006). Under space reduction conditions, captive bonobos engage in a high number of play faces (Tacconi and Palagi, 2009). Playing in limited spaces (i.e., indoor areas), where the escape opportunities are reduced, implies a higher level of social tension that affects the out-come of the session. The presence of clear signals conveying a positive intent is necessary to downgrade the arousal possibly induced by crowding conditions and/or the presence of an audience (van Hooff, 1989; Judge and de Waal, 1993; Flack et al., 2004; Cordoni and Palagi, 2007; Palagi et al., 2007). Considering the enlarged group association of wild geladas made up of familiar and less familiar subjects, it is difficult to understand if the redundant use of play faces during inter-OMU play is due to the presence of adults of other OMUs or is simply linked to the necessity to manage particularly asymmetric and risky sessions. Given that the performance of lip-smacking, a context-independent signal (Palagi and Mancini, 2011), was not predicted by the group membership of the players involved, we can hypothesize that during the play, the context-specific signals (i.e. the play face) are more sensitive to the variability of each play session both in terms of player familiarity and play modality (Palagi and Mancini, 2011; Gallo et al., 2021) (Prediction 1b not supported).

Although lip-smacking did not seem to be sensitive to either the familiarity shared by the players or their play modality, it has an important role in enhancing the RFM phenomenon. Indeed, we found that when players displayed lip-smacking, the play faces were mimicked more frequently (Fig. 3a). In this view, by expressing affiliative motivation, lip-smacking seems to have an indirect role in enhancing play communication. It is worth noting that the incidence of RFM was obtained by normalizing the RFM events on the number of play faces performed in each session. Such analytical approach makes the calculation of RFM incidence independent from the number of play faces displayed by the players. This allows a precise evaluation of the entity of the phenomenon that can be investigated without any bias due to the length of the playful interaction and, consequently, to the total number of playful facial expressions performed.

We did not find any evidence of a role of group membership in shaping the mimicry phenomenon. RFM appears to be strictly associated to the cooperative modality of the session more than to players' familiarity: the more symmetric the session, the higher the incidence of RFM (Fig. 3b) (prediction 2a not supported). There is evidence that RFM can help synchronize motor actions between players both in human and non-human animals (Palagi et al., 2020; Nieuwburg et al., 2021). The linkage between RFM and play equilibrium is particularly evident in tolerant (e.g., *Macaca tonkeana,* Scopa and Palagi, 2016) and cooperative species (e.g., *Suricata suricatta,* Palagi et al., 2019a) whose relation-ships, not relying on formalized hierarchical behaviors, need flexible communicative modules to manage and negotiate fluid social interactions, including play (Freeberg et al., 2012; Kavanagh et al., 2021).

Our results also show the effects of communicative signals on the length of playful interactions. We found that the duration of each session was positively predicted by both the presence of lip-smacking (Table 1c; Fig. 4b) and the incidence of RFM (Fig. 4a) (prediction 2c supported). The recruitment of LS from other affiliative contexts seems to reinforce the playful mood, enhance the cooperation among players (Scopa and Palagi, 2016) and, consequently, increase play duration. During an RFM interaction, the subjects must be attentive to the playmate's face, perceive the facial expression and correctly decode the information that such expression conveys (Provine, 1996, 2004; Palagi, 2018). In this perspective, the presence of RFM phenomenon can improve the communication among players and promote the behavioral coordination that leads to prolonged interactions (Pellis and Pellis, 2009; Palagi and Mancini, 2011). Our finding is in line with the previous evidence on captive geladas (Mancini et al., 2013b) and Tonkean macaques (Scopa and Palagi, 2016) demonstrating that RFM is a tool to increase the duration of play sessions. Prolonging playful interactions maximizes the benefits of this activity by allowing the players to improve their motor and social competence under a safe behavioral context (Bekoff and Byers ,1981; Pellegrini and Smith, 1998; Pellis and Iwaniuk, 2000; Nunes et al., 2004; Bekoff and Pierce, 2009; Pellis et al., 2010a, b; Nolfo et al., 2021).

In conclusion, the importance of RFM in modulating play fighting is unveiled in geladas considering their peculiar social organization (Snyder-Mackler et al., 2012). Play fighting is the only positive direct social interaction that can involve subjects of different OMUs (Dunbar and Dunbar, 1975). Therefore, a sophisticated communication system such as RFM can create a 'safe environment' for the players of both the same and different OMUs. We should also consider that immature play fighting occurs in a strict proximity of adults of different OMUs (audience). In this perspective, further exploration on the audience effect on the incidence of both play faces and RFM would be necessary to verify if such an exchange of signals strategically conveys information to third parties or is simply the byproduct of the emotional arousal experienced by wild geladas during play.

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Data availability

The dataset used for the analyses is provided as supplementary material.

Declarations

Ethics approval This study was approved by University of Pisa (Animal Care and Use Board) (Italy), the University of Turin (Italy) and the Ethiopian Wildlife Conservation Authority (EWCA). Since the study was purely observational, the committee waived the need for a permit. The study was conducted with no manipulation of animals. All applicable international, national and/or institutional guidelines for the use of animals were followed.

Conflict of interest

The authors declare no competing interests.

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Supporting material – Tables

Table S1

Categorization of play behavioral patterns as advantageous, disadvantageous, and neutral that have been used to calculate the Play Asymmetry Index (PAI). Play signals were not included in the PAI calculation. The behavioral patterns were obtained from both the ethograms of Dunbar (1975) and Palagi and Mancini (2011) and the observations of the present study. The categorization follows the criteria adopted in Gallo et al. (2021).

BEHAVIOUR	**DESCRIPTION**
ADVANTAGEOUS BEHAVIOURS	
social jump	An animal jumps on the playmate
play recovery a thing	An animal chases a playmate and attempts to grab an object carried by it
play push down	An animal attempts to push down the playmate from an element present in the environment
climb or stand on another	An animal climbs or stands on the body of a conspecific
play mount	An animal attempts to mount the playmate
play run	An animal rapidly chasing the playmate
play retrieve	An animal blocking a playmate to avoid its flight
play pull	An animal grasps another playmate

play kick	An animal kicks the playmates with its feet
play bite	An animal bites gently the playmate’s body
play slap	An animal slaps any part of the playmate’s body
play push	An animal pushes another playmate with its hands
DISADVANTAGEOUS BEHAVIOURS	
Roll	Turning the body from side to side while supine; can be complete or incomplete rotation
Somersault	Turning the body one full rotation head over heels
Pirouetting	An animal performs one or numerous rotations of the body by using one of its feet as keystone
play crouching	A position where the knees are bent and the upper bodyis down lower to the ground
moon walking	An animal walking to backwards
NEUTRAL BEHAVIOURS	
Jump	An animal jumps on the ground or on the elementpresent in its environment
slide down	An animal slides down from an element in theenvironment
object play	An animal holds and manipulates an object
mouth wrestling	rotational movement of the heads with open mouth whilesitting or laying positions
rough and tumble	Vigorous wrestling typically consisted of

	chasing, lunging, tackling, falling on the other, and vigorous mock biting. R&T can be characterized by a rapid sequence of 'advantageous' and 'disadvantageous' motor actions sometimes difficult to carefully discern. Since it was not always possible to clearly categorize each pattern forming a R&T session and assign with certainty any kind of directionality to the actions, we considered the sequence as neutral.
Leapfrog	An animal jumps above the head of its playmate during a movement
tug of war	Two animals doing a tug of war with an object

CHAPTER 4

Yawn contagion in bonobos: Another group, another story

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Yawn contagion in bonobos: another group, another story

Running title: New elements on bonobo yawn contagion

Ivan Norscia[a*], Marta Caselli[a], Gabriele De Meo[a], Giada Cordoni[a], Jean-Pascal Guéry[b], Elisa Demuru[c,d]

[a]Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy
[b]La Vallée des Singes, 86700, Romagne, France
[c]Dynamique Du Langage, CNRS-UMR 5596, University Lyon 2, Lyon, France
[d]Equipe de Neuro-Ethologie Sensorielle, ENES/CRNL, CNRS-UMR 5292, Inserm UMR S1028, University of Lyon/Saint-Etienne, Saint-Etienne, France

*Corresponding author: Ivan Norscia[a], Department of Life Sciences and Systems Biology, University of Torino, via Accademia Albertina 13, 10123, Torino, Italy.
Email: ivan.norscia@unito.it Tel: +39 011 6704547

Abstract

In primates, yawn contagion (the yawning response elicited by others' yawn) is variably influenced by individual (e.g., sex, age) and social factors (e.g., familiarity) and possibly linked to interindividual synchronization, coordination, and emotional contagion. Two out of three studies on yawn contagion in bonobos (*Pan paniscus*), found the presence of the phenomenon with mixed results concerning the effect of familiarity and no replication on its modulating factors. To address this puzzling issue, we recorded all occurrences data on yawn contagion in a captive bonobo group (March-June 2021; 18 individuals; La Vallée des Singes, France). Contrary to chimpanzees and humans, the number of triggering yawns increased contagion, possibly owing to a higher stimulus threshold. This aspect may explain the interindividual variability observed in yawn contagion rates. In subjects under weaning, we did not detect yawn contagion and, as it occurs in certain human cohorts, yawn contagion declined with age, possibly due to reduced sensitivity to others. Females responded more than males and elicited more responses from females when showing sexual swelling. As reproductive females are central in bonobo society, our results support the hypothesis that -as in other Hominini- the most influential sex can influence yawn contagion. The relationship quality (measured via grooming/ play) did not affect yawn contagion, possibly due to bonobos' xenophilic nature. Overall, this study confirms the presence of yawn contagion in bonobos and introduces new elements on its modulating factors, pointing toward the necessity of cross-group studies.

Keywords: Apes, Emotional contagion, Hominini, *Pan paniscus*, Physiological synchronization

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Introduction

While spontaneous yawning is not dependent on the detection of others' yawns, contagious yawning occurs when the yawn emitted by an individual (hereafter trigger) works as a releasing stimulus (*sensu* Tinbergen and Perdeck, 1950) and induces yawning in another individual (hereafter responder) (Provine, 1989). Spontaneous yawning (or a yawning-like morphological pattern) is likely a plesiomorphic display because it is present in a wide range of vertebrates (Baenninger, 1987), including human (*Homo sapiens*, Provine, 1986, 2012) and non-human primates (Anderson, 2020).

Contagious yawning between conspecifics is possibly an apomorphic phenomenon described so far in a limited array of species (Palagi et al., 2020). From an adaptive point of view, yawn contagion can promote synchronization and coordination of activities within social groups (Palagi et al., 2020). Moreover, it can be the expression of interindividual physiological resonance (Prochazkova and Kret, 2017) and possibly emotional contagion, a powerful driver of prosocial behavior (de Waal and Preston, 2017).

Experimental and naturalistic studies on chimpanzees (*Pan troglodytes*) (e.g., Anderson et al., 2004; Campbell and de Waal, 2011; Campbell and Cox, 2019) and humans (e.g., Provine, 1986, 1989; Norscia and Palagi, 2011; Bartholomew and Cirulli, 2014; Chan and Tseng, 2017; Norscia et al., 2021a) have consistently found intraspecific yawn contagion. In bonobos (*Pan paniscus*) the situation is not as much clear. Amici et al. (2014) examined whether yawning was subject to response facilitation triggered by videorecorded yawns from conspecifics. They found that chimpanzees (14 subjects) but not bonobos (4 subjects) yawned significantly more while or after watching a familiar conspecific yawning on video. On the other hand, on a larger sample (25 subjects), Tan et al. (2017) found that bonobos showed evidence for involuntary, contagious yawning in response to videos of yawning conspecifics. Finally, Demuru and Palagi (2012) also reported yawn contagion in captive bonobos (12 subjects) based on ethological observations under naturalistic conditions. Hence, yawn contagion as a social signal might have been present in the last common ancestor between *Pan* and *Homo*.

Beyond Hominini, it is not possible to associate the emergence of yawn contagion with a single common ancestor. Yawn contagion was not detected in lowland gorillas (*Gorilla gorilla gorilla*; Amici et al., 2014; Palagi et al., 2019) but it was found in orangutans (*Pongo* spp.; van Berlo et al., 2020) which separated earlier from the human line (Groves, 2018). Interestingly, lowland gorillas show low affiliation levels (Palagi et al., 2019) whereas orangutans do not form social groups, but orangutans might have been more social in the past (Harrison and Chivers, 2007). In non-human primates, yawn contagion studies show mixed results (cf. geladas, *Theropithecus gelada*, Palagi et al., 2009; Gallo et al., 2021; Tonkean macaque, *Macaca tonkeana*, Palagi and Norscia, 2019; but see: stump-tailed macaques, *Macaca arctoides*: Paukner and Anderson, 2006; Japanese macaque, *Macaca fuscata*, Palagi and Norscia, 2019). Finally, no evidence of yawn contagion was found in strepsirrhines (*Lemur catta* and *Varecia variegata*, Reddy et al., 2016) even though contagious yawning is present in non-primates (Gallup et al., 2015; for review: Palagi et al., 2020). Hence, yawning might have been co-opted as a communicative signal multiple times over the course of the evolution, in relation to the type of sociality.

When present, yawn contagion in primates usually occurs in the few minutes following the yawning stimulus (hereafter triggering yawn) with a peak in the first minute in Hominini (e.g., humans: Palagi et al., 2014; chimpanzees, *Pan troglodytes*: Campbell and Cox, 2019; and bonobos: Demuru and Palagi, 2012). In humans, perceptual factors may influence the yawning response probability (Massen and Gallup, 2017; Norscia et al., 2020). However, the distance between trigger and responder and/or the number of observed triggering yawns were not found to affect yawn contagion (humans: Norscia and Palagi, 2011; chimpanzees: Campbell and Cox, 2019; geladas: Palagi et al., 2009).

Yawn contagion can be influenced by individual and social factors (Palagi et al., 2020). The age of the responder can affect yawn contagion rates in some cohorts of humans (Anderson and Meno, 2003; Helt et al., 2010; Hoogenhout et al., 2013; Bartholomew and Cirulli, 2014) and chimpanzees (Madsen et al., 2013).

No study so far has addressed this issue in bonobos. Moreover, in Hominini the yawning response can vary depending on the sex of the responder or the trigger. For example, women may respond more to others' yawns (Norscia et al., 2016a, 2016b; Chan and Tseng, 2017), although this does not occur in all cohorts (Norscia and Palagi, 2011; Bartholomew and Cirulli, 2014). Moreover, in the *Pan* genus, yawning response can vary in relation to the trigger's sex, possibly depending on the social role that each sex has in different species (Demuru and Palagi, 2012; Massen and Gallup, 2017). Finally, yawn contagion was found to be influenced by the level of familiarity between subjects in humans (Norscia et al., 2020; Norscia and Palagi, 2011), chimpanzees (Campbell and de Waal, 2011), and in one out of two groups of bonobos (cf. Demuru and Palagi, 2012; Tan et al., 2017), with highest yawn contagion rates being recorded between particularly familiar subjects.

In sum, two out of the three independent studies on the presence of yawn contagion in bonobos detected the phenomenon (cf. Demuru and Palagi, 2012; Amici et al., 2014; Tan et al., 2017) and yawn contagion was higher between closely bonded (compared to weakly bonded) group mates (Demuru and Palagi, 2012) but not between group mates when compared to non-group mates (Tan et al., 2017). To better understand the phenomenon, we investigated yawn contagion in yet another group of bonobos. We formulated the following predictions.

Prediction 1: Presence and distribution of yawn contagion- Based on previous findings on the presence of yawn contagion in two bonobo groups (Demuru and Palagi, 2012; Tan et al., 2017), we expected to find the phenomenon also in our study group (Prediction 1a). Demuru and Palagi (2012) found the maximum yawn contagion rates in the first minute after the triggering stimulus. Hence, we expected to find a similar result in our study group (Prediction 1b). Because yawn contagion was not found in all bonobos (Amici et al., 2014), we expected to find a high contagion variability across subjects (Prediction 1c).

Prediction 2: Perceptual factors- Possibly due to the high visual acuity of anthropoid primates (Fleagle, 2013), the spatial distance from trigger and responder was found to have no effect on yawn contagion in chimpanzees (Campbell and Cox, 2019) and geladas (Palagi et al., 2009). Hence, we expected to find no influence of trigger-responder distance on yawn contagion in bonobos (Prediction 2a). Moreover, in humans and chimpanzees observing several yawns in a row does not seem to raise the chance of yawn contagion (humans: Norscia and Palagi, 2011; chimpanzees: Campbell and Cox, 2019). Hence, we expected a similar result in bonobos owing to their phylogenetic closeness with humans and chimpanzees (Prediction 2b).

Prediction 3: Individual and social factors- In the Hominini, the trigger's rank and sex can have an influence on yawn contagion rates, with individuals responding mostly to men in certain cohorts of humans (for yawns that are heard but not seen; Norscia et al., 2020) and chimpanzees (dominant males especially;

Massen and Gallup, 2017) and to females in bonobos (Demuru and Palagi, 2012). While males are central in chimpanzee dominance relationships (Bray et al., 2021; Lewis et al., 2021), in bonobos reproductive females are central in determining group dynamics (e.g., Furuichi, 2011). Hence, we expected that trigger's rank and sex -especially adult females- could play a major role in eliciting the yawning response (Prediction 3a). As concerns the effect of age, no study on bonobos has addressed this factor on yawn contagion so far. However, age appears to have an effect in humans (Anderson and Meno, 2003; Helt et al., 2010; Bartholomew and Cirulli, 2014) and in chimpanzees (Madsen et al., 2013), with yawn contagion being higher in adults than in immature subjects. In certain cohorts of adult humans, yawning decreases with aging (Bartholomew and Cirulli, 2014). This aspect has not been investigated in chimpanzees. Owing to the phylogenetic closeness of bonobos to humans and chimpanzees (Prüfer et al., 2012), we expected that age might have a similar effect on yawn contagion in our study group (Prediction 3b). In humans and chimpanzees, familiarity between individuals has been reported to increase yawn contagion rates (humans: Norscia and Palagi, 2011; Norscia et al., 2016a; chimpanzees: Campbell and de Waal, 2011). In bonobos, no familiarity effect was found between non-group members in an experimental setting (using video trials; Tan et al., 2017) but it was found within known subjects in naturalistic conditions, with yawn contagion being highest between closely bonded group mates (Demuru and Palagi, 2012). Thus, we expected to find a positive effect of familiarity on yawn contagion in our bonobo group, observed under naturalistic conditions (Prediction 3c).

Methods

Study site and group

The bonobo study group was housed at La Vallée des Singes (Romagne, France) with no fission-fusion management. During the day, the subjects could move freely from the indoor enclosure (500 m^2) to a wooded external island (1 ha), except in case of bad weather (in which case when they were kept indoors). The group was composed of 18 individuals (age range: from 0 to 53 years; mean±SE: 16.722±3.035) including adults (4 males and 7 females; age: ≥12 years); juveniles (2 males and 3 females; age: 6-9 years); one weaning female (4 years old); and one lactating newborn male (4 months when the study started). Maternal kinship was known whereas paternal kinship was not known for all individuals (Full group info: Table S1).

Data collection

Behavioral data were collected via audio-recordings by two observers (M.C. and G.D.M.) on a daily basis from March to June 2021 (8:30-13:00 or 13:00-17:30; observation hours/individual, mean±SE: 66.30±3.78). Data on grooming, contact sitting and social play (especially present in immatures) were used to determine dyadic affiliation levels and were collected via 10-min scan sampling (Altmann, 1974). Data on agonistic patterns (including displacements, avoidance, priority on food access, overt aggression, etc.) were gathered via all occurrences sampling method (Altmann, 1974) (full ethogram: Table S2).

Bonobo females show a conspicuous sexual swelling (increased anogenital area turgidity) that follows a cycle of roughly 40 days and is not strictly associated with ovulation (Dixson, 1983; Douglas et al., 2016). Data on individual sexual swelling cycle were collected by zookeepers on a specific data sheet, where they indicated whether a female had the swelling cycle (from minimum to maximum size turgidity) or not (menopause: one female; lactation: one female; contraceptive: one female; juveniles females: two). Yawning bouts were collected via the all-occurrences sampling method (Altman, 1974) in absence of external perturbing events (e.g., aggression, food distribution; 595 yawns collected in total). The yawning pattern involved mouth opening, with inhalation and a more rapid closing and exhalation (Baenninger, 1997). No yawn was vocalized (via the use of vocal folds). For each yawn (triggering yawn) emitted by a subject, we recorded: (i) identity, sex and age of the yawner (trigger); (ii) identity, sex and age of all the subjects that were visible to the human observer and that could see the triggering yawn (potential responders); (iii) time of the triggering yawn (time of last consecutive yawn if more yawns were emitted in a row); (iv) distance between each potential responder and the last trigger (≤1 m, 1 < distance ≤ 10 m, > 10 m). A yawn contagion sequence is shown in Figure 1 and Video S1.

Figure 1 Yawn contagion sequence. Yuli (7yo female) emits a yawn and Lokoro (6yo male) responds after 18 s. Lokoro is sitting within 1 m from Yuli and can see the triggering yawn

Within the 5-min time window in which a yawning response to a triggering yawn may be observed (Provine, 1989; Palagi et al., 2009), we selected a 3-min time window to check for the yawning response. We did so as in the fourth minute there is the highest probability of autocorrelation (a yawn performed by a subject at t0 increases the probability to have another yawn by the same subject at t(0+X) where X is the increasing unit of time; Kapitány and Nielsen, 2017). To further reduce autocorrelation issues, in case of a yawning chain (i.e., several yawns emitted in a row by the same subject during 3 min, with no other subject yawning), we considered as a response only the first yawn emitted after the last triggering yawn (Gallo et al., 2021). When more than one yawning response occurred from different subjects, the first responder would become a trigger and we noted whether -for each responder- the triggering stimulus came from one or multiple triggers within the fixed time window. For each minute within the 3-min time window, we recorded the time of each yawning response (if any), so as to determine -a posteriori- the minute in which such response occurred (first/second/third) and the identity of the responder. We did not include in the yawning response dataset the subjects (i.e., the potential responders) that had their head rotated by 180° with respect to the trigger or when a physical, sight-blocking obstacle prevented them from seeing the trigger.

Operational definitions and interobserver reliability

Agonistic encounters, spanning overt aggression and less invasive competitive interactions (e.g., displacements, avoidance, food priority), between individuals were defined as “decided” if a winner and a loser were clearly recognizable and as “undecided” if not. In particular, an individual was considered the loser when they fled, screamed, left the food or the place to the other subject, or emitted submissive vocalizations and/or showed submissive facial expressions (Table S2).

Bonobos showed a strong yawn contagion peak in the first minute after the triggering stimulus in a previous study (Demuru and Palagi, 2012). Thus, we checked for the presence of yawn contagion in each minute of the selected time window. We applied a modified version of the Post-Conflict/Matched Control

(PC-MC) method, initially designed to check for post-conflict reunions in animals (de Waal and Yoshihara, 1983) and recently applied to check for grooming contagion (Berthier and Semple, 2018; Ostner et al., 2021) and for the association between spontaneous yawning and behavioral transitions or stressful events (Zannella et al., 2015). In particular, in our case we identified two conditions: (1) Post-Yawning (PY)- after the last triggering yawn a potential responder was observed in a 3-min time window to record whether and when (first, second or third PY minute) there was a yawning response; (2) Matched Control (MC)- at the same time (±1 h) as the PY in the first suitable day, under similar social and environmental conditions (e.g., same weather, presence of other subjects) and in the absence of any previous triggering yawn, the same potential responder was observed for three minutes to check whether and when (first, second or third MC minute) yawning occurred.

For each minute, PY-MC pairs were defined as: (i) attracted (APs) if the yawn occurred in the selected minute in PY and not in MC or if it occurred in PY in a previous minute compared to MC; (ii) dispersed (DPs) if the yawn occurred in the selected minute in MC and not in PY or if it occurred in PY in a following minute compared to MC; (iii) neutral (NPs) if the yawn occurred in the selected minute both in PY and in MC or if it did not occur at all in both conditions.

Based on the method of calculation of the Corrected Conciliatory Tendency (de Waal and Yoshihara, 1983; Veenema et al., 1994) for post-conflict management, we calculated the Individual Contagion Tendencies (ICTs) as follows:

$$\frac{(APs - DPs)}{(APs + DPs + NPs)}$$

The interobserver reliability between the two data collectors (M.C. and G.D.M.) was calculated via Cohen's k on 10% of the yawning events which they recorded concurrently and independently. Cohen's k was calculated for all the variables considered (yawner identity, possible responder identity, detection condition of the possible responder, distance, yawning response and minute) and was always higher than 0.85 (level of agreement: strong, *sensu* McHugh, 2012).

Statistical elaboration

We determined the individual ranking position and hierarchy steepness based on decided agonistic interactions (ethogram: Table S2) via Normalized David's Scores (NDS; de Vries et al., 2006). NDS were individually assessed via an aggression sociomatrix including the number of decided agonistic encounters/dyad (R ‘steepness’ package; CRAN.R-project.org/package=steepness). Our study group showed a relatively low steepness (0.425), which indicates a rather shallow hierarchy. Further details provided in appendix 1.

Via the freeware Gephi 0.9.2 (www.gephi.org/; dual license CDDL 1.0 and GNU General Public License v3), we obtained the social network of yawn contagion (Figure 2). It includes individuals (nodes) and interindividual connections (directed edges) derived from the number of directional dyadic contagion events (AB if A was the trigger and B the responder; BA if the other way around) normalized over the number of yawns to which the responder was exposed in the 1-min time-slot). The node size is based on in-degree centrality (or prestige) that in our case is the frequency of yawning stimuli received and responded to by a node (sensitivity to contagion; Golbeck, 2013; Saqr et al., 2018). Further details are reported in Appendix 1.

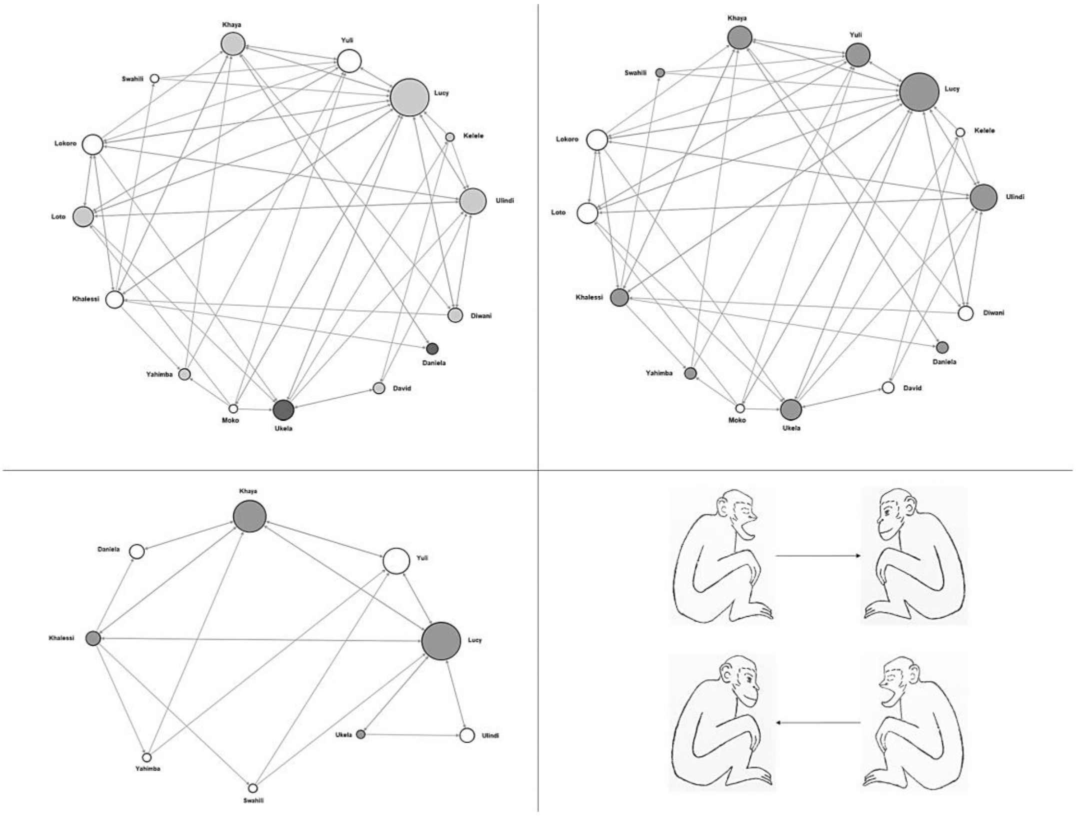

Figure 2 Yawn contagion network. Node size is based on the in-degree prestige. The different quadrants highlight (top-left) age classes (<12 years old: white nodes; 12–30 years old: light grey nodes; over 30: dark grey nodes); (top-right) sex (males: white nodes; females: gray nodes); (bottom-left) swelling status within the female network (females without swelling: white nodes; females with swelling: grey nodes). Edge arrows (bottom-right) indicate the direction of contagion between nodes and go from the trigger to the responder

Yawn contagion was never observed in the very few bouts collected on the newborn (which was rarely in a position that allowed reliable yawning detection) and in the 4-year-old infant (which would often stay with the mother in non-observable zones of the enclosure). Therefore, only juveniles (6-7 years old), subadult (9 years old) and adult (≥12 years old) subjects were included in the analyses (N=16 subjects).

To check whether yawn contagion was present (non-normal data distribution: Kolmogorov–Smirnov, $N_{individuals}=15$, $0.895 \leq Z \leq 21.833$, $P<0.05$) we applied the non-parametric Wilcoxon's pair test to compare the number of attracted versus dispersed pairs at the individual level in each of the three minutes following the triggering stimulus (number of APs > number of DPs). To compare the yawning response level after a triggering stimulus (PY) and in absence of yawning stimuli (MC) we applied a parametric paired t test for the first and the second minute (normal data distribution: Kolmogorov–Smirnov, $N_{individuals}=15$, $0.943 \leq Z \leq 1.284$; P= n.s.) and the Wilcoxon's pair test for the third minute (non-normal distribution: Kolmogorov–Smirnov, $N_{individuals}=15$, Z=1.833/1.992; $P<0.05$). Due to the normal distribution of the variables (Kolmogorov–Smirnov, $N_{adults\_subtadults}=15$, $Z \geq 0.501$, P= n.s.), Pearson's bivariate correlation test was used to correlate NDSs and ICTs. To check whether there was a significant variation in the ICTs across subjects, within the group, we applied a one-sample t test (Kolmogorov–Smirnov, $N_{individuals}=15$, Z=0.710, P= n.s.). Based on the PC-MC method (e.g., Schino et al., 1998), in the previous analysis we included subjects that had at least three PY occasions, so that they could have one pair per type (AP, DP, NP; min PY-MC pair number for the other subjects: nine).

Because around 85% of the yawning responses occurred in the first minute from the triggering yawn, we verified what factors could affect the yawning response occurring in the 1-min time slot following the last yawning stimulus from another subject (triggering yawn). To this purpose, we ran two different Generalized Linear Mixed Models ($GLMM_1$ and $GLMM_2$) on the cases where the yawning stimulus came from a single trigger in the previous minute. In both models, we included the presence/ absence of the yawning response as the dependent, binary target variable (presence=1; absence=0).

In $GLMM_1$ (N_{cases} = 344), the following fixed factors were included: (i) triggering yawn number (factor; 1=one yawn; 2=two yawns; 3=more than three yawns); (ii) distance (factor: 1=individuals within 1 m; 2=from 1 to 10 m; 3=more than 10 m); (iii) sex of the trigger and potential responder (factor; M=male; F=female); (iv) age of the trigger and potential responder (numeric; years); (v) trigger's and responder's rank (numeric; NDS); (vi) affiliation levels (numeric; hourly frequencies with data normalized over the observation time). In $GLMM_2$ (N_{cases}=133), run on female dyads only -to check whether the swelling status of trigger and/or responder would affect yawn contagion- the following fixed factors were included: trigger's reproductive state and responder's swelling status (factor;

0=without sexual swelling; 1=with sexual swelling). In both $GLMM_1$ and $GLMM_2$ the combination between trigger and potential responder's identity (dyad) was included as a random factor.

The GLMMs were fitted in R (R Core Team, 2018; version 3.5.3) by using the function “glmer” of the R-package *lme4* (Bates et al., 2015). As a first step we verified if the full model significantly differed from the null model that included the random factors only (Forstmeier and Schielzeth, 2011). The likelihood ratio test (Dobson and Barnett, 2018) was used to test this significance (analysis of variance 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 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., 2010) 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 numeric variables of $GLMM_1$ via the “vif” function in R. All VIF values were between 1 and 2 (min-max range: 1.29-1.48), thus indicating no collinearity. We calculated the effect size via the package *effectsize*, function effectsize which returns the best effect-size measure for the provided input GLMM.

Results

Presence and distribution of yawn contagion

Across the three minutes following a triggering yawn, yawn contagion was present in the first minute, but not in the second and in the third minute. The number of attracted pairs was significantly higher than the number of dispersed pairs in the first minute (Wilcoxon's paired test: $N_{individuals}=15$, $T=3.50$, $P=0.001$), but not in the second minute (nonsignificant trend; Wilcoxon's paired test: $N_{individuals}=15$, $T=12$, $P=0.053$) and in the third minute (Wilcoxon's paired test: $N_{individuals}=15$, $T=0.00$, $P=0.102$) (Figure 3a). Consistently, the level of yawning after a triggering yawn (PY condition) was significantly higher than the level of baseline yawning (MC condition) in the first minute (paired t test: $N_{individuals}=15$, $T=3.826$, $df=14$, $P=0.002$; mean±SE, PY=7.80±1.93, MC=1.87±0.61), but not in the following minutes (paired t test; 2 min: $N_{individuals}=15$, $T=1.871$, $df=14$, $P=0.082$; mean±SE, PY=1.27±0.30, MC=0.67±0.33; Wilcoxon's paired test: 3 min: $N_{individuals}=15$, $T=1.50$, $P=0.414$; mean±SE, PY=0.27±0.15, MC=0.13±0.39; Figure 3b).

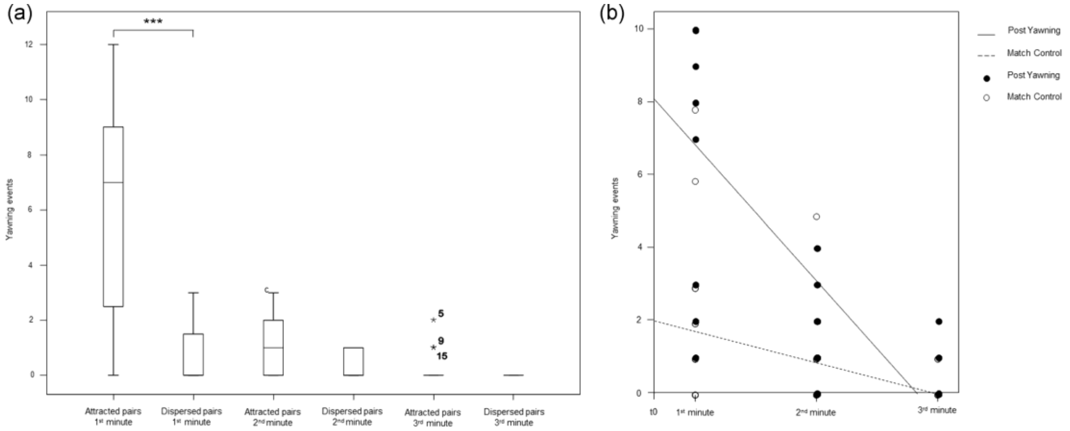

Figure 3 a Differences between attracted and dispersed pairs in the 1st, 2nd, and 3rd minute after the yawning stimulus. Solid horizontal lines: medians; box length: interquartile range; thin horizontal lines: observed value range; asterisks: probability level: *p < 0.05; **p < 0.01, ***p < 0.001. **b** Dispersion plot with regression lines showing the decrease of contagious (PY) and spontaneous yawning (MC) in the 3 min following the yawning stimulus

Within the study group, there was a significant variation in the ICT across individuals (mean±SE: 0.255±0.0423; one-sample t test: $N_{individuals}$=15, T=5.989, *df*=14, P<0.001) with one adult male showing no contagion (yawning rate in MC>PY). Figure 2 shows the yawn contagion network and the different parts of the figure high-lights different features of the nodes (Figure 2a: age; Figure 2b: sex; Figure 2c: swelling condition).

Variable affecting yawn contagion

The full model ($GLMM_1$; target variable: yawning response) including all fixed factors (trigger's and responder's NDS, trigger's yawn number, distance from the trigger, trigger's and responder's sex and age, social bond) significantly differed from the null model only including the random factor (trigger-responder identity dyad) (likelihood ratio test: χ^2=26.454, *df*=11, P=0.006). As at least one predictor had a significant effect on the response, we moved on with a "*drop1*" procedure. We found that the trigger yawn number had a significant effect on the yawning response, which was higher as the number of triggering yawns was ≥ three (Table 1; Figure 4a; Tukey test: 1 *vs*. 2 yawns, Est=0.376, SE=0.350, Z=1.076, P=0.514; 1 *vs*. ≥3 yawns, Est=1.786, SE=0.636, Z=2.822, P=0.012; 2 *vs*. ≥3 yawns, Est=1.409, SE=0.693, Z=2.034, P=0.097). Moreover, the responder's sex had a strong significant effect on the yawning response (Table 1), with females being more likely than males to yawn after perceiving a triggering yawn (Figure 4b). Finally, the responder's age also had a significant effect, with yawn contagion decreasing with age (Table 1 and Figure 4c). No other factors had a significant effect on the target variable (see Table 1 for full results).

Table 1 Influence of individual, perceptual, social factors (GLMM$_1$), and female swelling status (GLMM$_2$) on yawn contagion

Predictors	Estimates	SEM	CI$_{95}$	Effect size	χ^2	P
GLMM$_1$	N$_{cases}$=344; Full vs. null model: χ^2=26.454; *df*=11; P=0.006					
(Intercept)[a]	-1.640	0.845	-2.70, -0.86	a	a	a
NDS trigger	-0.017	0.084	-0.35, 0.29	0.03	-0.198	0.843
NDS responder	0.120	0.081	-0.08, 0.60	0.26	1.488	0.137
Trigger yawn number (two yawns)[b]	0.376	0.350	-0.31, 1.06	0.38	1.076	0.282
Trigger yawn number	1.786	0.633	0.55, 3.03	1.79	2.822	**0.005**

(more than three yawns)[b]						
Affiliation levels	-0.829	1.429	-0.34, 0.18	0.08	-0.580	0.562
Distance (from 1 to 10m)[b]	0.040	0.382	-0.71, 0.79	0.04	0.103	0.918
Distance (more than 10m)[b]	0.090	0.533	-0.95, 1.13	0.09	0.169	0.865
Trigger sex (female)[b]	0.021	0.292	-0.55, 0.59	0.02	0.072	0.942
Responder sex (female)[b]	0.945	0.290	0.38, 1.51	0.94	3.263	**0.001**
Trigger age	-0.008	0.016	-0.44, 0.25	0.09	-0.520	0.603
Responder age	-0.038	0.018	-0.82, -0.04	0.43	-2.140	**0.032**
GLMM$_2$	N_{cases}=133; Full *vs.* null model: χ^2=6.668; *df*=2; P=0.036					
(Intercept)[a]	-1.424	0.391	-2.19, -0.66	1.42	a	a
Trigger swelling status (with sexual swelling)[c]	0.947	0.389	0.18, 1.71	0.95	2.434	**0.015**
Responder swelling status (with sexual swelling)[c]	0.248	0.394	-0.52, 1.02	0.25	0.629	0.530

[a] Not shown as not having a meaningful interpretation

[b] These predictors were dummy-coded, with the reference category as follow: Trigger yawn number: “one yawn”; Social bond: “strong”; Distance: “within one meter”; Trigger sex: “male”; Responder sex: “male”

[c] These predictors were dummy-coded, with the reference category as follow: Trigger swelling status: “without sexual swelling”; Responder swelling status: “without sexual swelling”

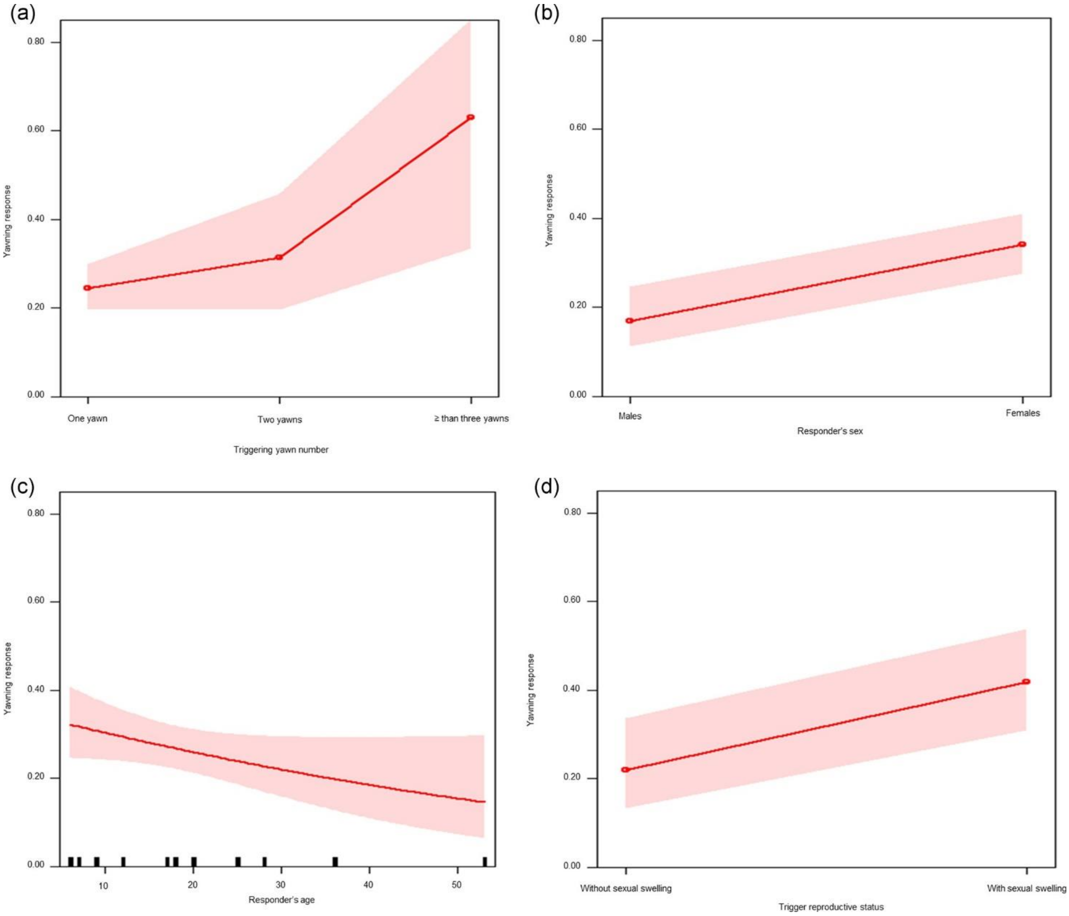

Figure 4 Effect plot of variables having a significant influence on the yawning response. The occurrence of yawning response (Y axis): **a** increases as the “number of trigger yawns” (X axis) increases, **b** varies according to the responder sex (X axis) and is highest in females; **c** decreases as the responder age (X axis) increases; **d** varies according to the trigger sex (X axis) within female-female dyads and is preferentially triggered by females with swelling. Band represents the confidence interval

The second full model ($GLMM_2$; target variable: yawning response) including all fixed factors (trigger reproductive status and responder reproductive status) significantly differed from the null model only including the random factor (trigger-responder identity dyad) (likelihood ratio test: $\chi^2=6.668$, $df=2$, $P=0.036$). As we found that at least one predictor had a significant effect on the response, we moved on with a "*drop1*" procedure. We found that the trigger's swelling status had a strong significant effect on the yawning response (Table 1), with the females showing a swelling cycle eliciting more yawns than those without sexual swelling cycle (i.e., in menopause, lactating or under contraceptives) (Figure 4d).

Discussion

Presence of yawn contagion

Yawn contagion was present in our study group because it was more likely that bonobos yawned after seeing a yawn (PY condition) compared to when they did not observe any previous yawn (MC condition; Prediction 1a supported; Figure 3). Hence, yawn contagion may be present at the population level, as it has been found so far in three different groups (present study; Demuru and Palagi, 2012; Tan et al., 2017). Yawn contagion is also present in different cohorts of other Hominini (chimpanzees: Anderson et al., 2004; Campbell and de Waal, 2011; Campbell and Cox, 2019; humans: e.g., Provine, 1989; Norscia and Palagi, 2011; Bartholomew and Cirulli, 2014; Chan and Tseng, 2017; Cordoni et al., 2021) and -as a form of autonomic contagion- can increase interindividual synchronization and coordination (de Waal and Preston, 2017; Prochazkova and Kret, 2017; Casetta et al., 2021).

We found that the phenomenon was present only in the first minute after the yawn stimulus (Prediction 1b supported), when we detected a significant difference between PY and MC conditions (Figure 3b). On the one hand, this result is in line with previous reports showing a peak of yawn contagion in the first minute -compared to following minutes- in bonobos (Demuru and Palagi, 2012) and the other Hominini (chimpanzees: Campbell and Cox, 2019; humans: Palagi et al., 2014). On the other hand, our result introduces an element of novelty because it shows that yawn contagion occurred only (not just maximally) in the first minute (or up to the second minute, if we consider the nonsignificant trend as the basis for further investigation).

In our naturalistic study we found significant variability in the ICTs across subjects (Prediction 2c supported) and one adult male did not show contagion (yawning more in MC than in PY condition). Even though previous studies did not specifically focus on yawn contagion interindividual variation (Demuru and Palagi, 2012; Amici et al., 2014; Tan et al., 2017), Amici et al. (2014) found that in four bonobos yawning was not triggered by video stimuli of yawning conspecifics. Interindividual variability may explain at least in part why contagion is not expressed in all subjects. In healthy humans, 40%-60% of subjects did not show yawn contagion under laboratory conditions (Provine, 1986, 1989; Platek et al., 2003) and susceptibility to others' yawns appears to be stable across contexts (Bartholomew and Cirulli, 2014). Analogously in bonobos, yawn contagion can be context independent (e.g., resting/relaxing vs social tension contexts; Demuru and Palagi, 2012). Future studies on interindividual fluctuations can shed light on within-population variability.

Perceptual factors affecting yawn contagion

The spatial distance between trigger and responder had no significant effect on yawn contagion (Table 1; Prediction 2a confirmed). Consistently, no influence of trigger-responder distance was found in chimpanzees (Campbell and Cox, 2019) and geladas (Palagi et al., 2009). This is not surprising because anthropoid primates possess high visual acuity and mainly rely on stereoscopic vision to orient themselves in the world (Fleagle, 2013).

In our study group, yawn contagion probability increased as the number of triggering yawns increased (Prediction 2b not supported; Figure 4a; Table 1). This is in contrast with the situation found in humans and chimpanzees, in which no such effect was found (Norscia and Palagi, 2011; Campbell and Cox, 2019). Interestingly, Norscia et al. (2021b) found that in domestic pigs both trigger-responder spatial distance and the number of (non-vocalized) yawning stimuli affected yawn contagion rates possibly due to the scarce visual acuity of the species. It is possible that bonobos -compared to humans- possess a higher yawn contagion threshold and that the yawning response is most likely primed after observing multiple yawns. This possibility may contribute to the interindividual variability observed in bonobo yawn contagion and might point towards possible neurobiological differences in stimulus processing. Future cross-species studies are necessary to clarify this issue.

Individual and social factors modulating yawn contagion

Compared to males, females were not overall more effective as triggers even though a previous study found that adult females tended to induce others' yawns more than males (Demuru and Palagi, 2012). This difference may be due to the fact that our female sample included females with and without a swelling cycle, which allowed us to test for this variable (not tested before). We found that females with a swelling cycle elicited more yawning responses from other females compared to females without swelling cycle (Table 1 and Figure 4d). In this respect, Prediction 3a can be at least partially confirmed. Swelling in bonobos is an important communicative signal not just for males but also for females (Demuru et al., 2020) and can contribute to determining their social status by favoring female-female socio-sexual interactions and alliances (Furuichi, 2011; Moscovice et al., 2019). Analogously, in chimpanzees -in which males form alliances to control resources (Bray et al., 2021; Lewis et al., 2021)- males seem to be most powerful in eliciting yawn contagion, especially if dominant (Massen and Gallup, 2017). Rank per se had no significant influence on yawn contagion in bonobos possibly due to the high tolerance level of the species (Hare and Kwetuenda, 2010; Furuichi, 2011). Indeed, in our bonobo group hierarchy showed relatively low steepness, which indicates rather shallow hierarchy. Interestingly, females showed the highest yawn contagion rates (Table 1 and Figure 4b), which may be related to their central role in bonobo groups. Such a role may require an enhanced sensitivity to social signals, such as yawning, which may favor interindividual synchronization and social cohesion. In humans, an increased yawning response of women has been observed in some cases (Norscia et al., 2016a; Chan and Tseng, 2017) but not in others (Norscia and Palagi, 2011; Bartholomew and Cirulli, 2014). The socio-cultural influence characterizing different human cohorts makes it hard to single out an unambiguous effect of gender on yawn contagion (Palagi et al., 2020).

We detected no yawn contagion (as responders) in the two infants (aged 4 months and 4 years old) and our statistical analysis on subadults and adults showed that yawn contagion decreased with age (Table 1 and Figure 4c; Prediction 3b confirmed). The responder's age seems to affect yawn contagion also in other Hominini. In chimpanzees yawn contagion was found in adult subjects, but absent in infant subjects (Madsen et al., 2013). In humans, yawn contagion is absent, reduced or differently age-modulated in infants (Anderson and Meno, 2003; Helt et al., 2010; Millen and Anderson, 2011; Cordoni et al., 2021). In human and non-human mammals, the increase of yawn contagion with age (especially from the immature phase to adulthood) has been associated with possible maturation of socio-cognitive abilities and/or neural pathways that decode social cues and with the ontogenetic variation in the ability to identify the internal states of others (Madsen and Persson, 2013; Cordoni et al., 2021; Norscia et al., 2021b).

In certain human cohorts, yawn contagion can decline with age (over 40; Bartholomew and Cirulli, 2014) possibly due to a decreased sensitivity to others' states (Palagi et al., 2020). Yawn contagion - possibly mediated by bottom-up cognitive processes (Palagi et al., 2020)- might also decrease with age as the result of the increased top-down mechanisms in emotional processing. Interestingly, in humans, aging seems to be associated with a switch from bottom-up to top-down processes in emotion appraisal (Reed and Carstensen, 2012; Petro et al., 2021). Further neuroethological studies are necessary to verify these hypotheses.

Finally, the affiliation levels between group mates (a social attachment indicator; Dunbar, 1991) did not affect the likelihood of yawn contagion (Table 1; Prediction 3c not confirmed). Social attachment (informed by affiliation levels, kinship and/or group membership) can increase yawn contagion rates (Palagi et al., 2020). Such effect has been observed in humans (Norscia and Palagi, 2011;

Norscia et al., 2016a), chimpanzees (Campbell and de Waal, 2011) and other mammals (e.g., domestic pigs, Norscia et al., 2021b; wolves, Romero et al., 2014). The presence of the so-called ‘familiarity bias' suggests that emotional contagion may influence the phenomenon of yawn contagion (de Waal and Preston, 2017). In bonobos, the situation is puzzling because no effect of group membership (group vs. non-group members) was experimentally found in one group (Tan et al., 2017) whereas a positive effect of social bond between group mates was found in another group via a naturalistic approach (affiliation rates and kinship were combined; Demuru and Palagi, 2012). At the very proximate level, the familiarity bias on yawn contagion may be dampened in our study colony by the fact that individuals had been together in the same group -with no fission- fusion management- for a long time (min-max range: 4-12 years). Affiliation rates occurring in the short term may not reliably inform on long-term familiarity. At the ultimate level, the xenophilic nature of bonobos (showing affiliation between group residents and non- residents, high intergroup tolerance and food sharing with strangers; Idani, 1991; Furuichi, 2011; Tan and Hare, 2013; Tan et al., 2017; Lucchesi et al., 2020) may have contributed to reducing the adaptive value of familiarity. The lack of familiarity bias was also found in an opposite situation. Particularly, van Berlo et al. (2020) found the presence of yawn contagion in captive orangutans with no effect of familiarity was detected. Wild orangutans do not live in social groups but show dispersed sociality (with occasional encounters). Here, the effect of familiarity may have a reduced adaptive significance because individuals do not form preferential social bonds or alliances. The opposite cases of bonobos (Demuru and Palagi, 2012; Tan et al., 2017; present study) and orangutans (van Berlo et al., 2020) converge in indicating that the familiarity bias may be related to interindividual cohesion (proximate level) and type of sociality (ultimate level). In contrast with previous reports (Joly-Mascheroni et al., 2008; Silva et al., 2012; Romero et al., 2013), a meta-analysis showed that familiarity seems not to affect interspecific yawn contagion between dogs and humans (Neilands et al., 2020). A similar approach could help disentangle the familiarity issue in bonobos, especially if by including data collected with the same methodologies on different colonies. Once again - owing to the differences observed across study groups and sites- we stress the importance of expanding the dataset on yawn contagion to account for intergroup differences and clarify what factors can modulate the phenomenon at the population level.

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Author contributions

Ivan Norscia: research resources; Ivan Norscia, Elisa Demuru, Marta Caselli: students' training for data collection; Ivan Norscia: conceived, wrote the manuscript and analyzed data; Gabriele De Meo and Marta Caselli: collected and sorted out data; Giada Cordoni, Marta Caselli, Elisa Demuru: revised the manuscript; Jean-Pascal Guéry: provided access to resources and facilities; supported data analyses and conceptualization.

Data availability statement

The study data are available from the corresponding author upon reasonable request.

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Appendix 1

Details on material and methods

Hierarchy and ranking position determination

We determined the bonobo ranking position based on decided conflicts, by using the Normalized David's Scores (NDS) (de Vries et al., 2006). Via the R 'steepness' package (https://CRAN.R-project.org/package=steepness), NDS were individually assessed by using decided agonistic encounters. The individual values (number of decided encounters normalized over the individual observation time) were entered in a sociomatrix. NDSs were calculated on the basis of a dyadic dominance index (Dij) in which the observed proportion of wins (Pij) is corrected for the chance occurrence of the observed outcome. The chance occurrence of the observed outcome is calculated on the basis of a binomial distribution with each subject having an equal chance of winning or losing in every agonistic encounter (de Vries et al., 2006). The hierarchy steepness resulted from the absolute slope of the straight line fitted to the normalized David's scores plotted against the subjects' ranks (de Vries et al., 2006).

Yawn contagion network

A social network can be modelled as a graph constructed from relational data and can be defined as a set of social entities, such as individuals, with some relationships or interactions between them (Tabassum et al., 2018). Via the freeware Gephi 0.9.2 (www.https://gephi.org/, distributed under the dual license CDDL 1.0 and GNU General Public License v3), we obtained the social network for an ‘immaterial’ inter-individual connection: yawn contagion. The network includes the individuals of the group (nodes) and the interactions between them (edges) and it was derived from frequency of yawn contagion, calculated as follows: number of directional dyadic contagion events (directed edges: AB if A was the trigger and B the responder; BA if the other way around) divided the number of contagion occasions (number of triggering yawns to which the responder was exposed and had the opportunity to respond within 1 minute). We entered the trigger of the behavioral stimulus as source and the responder as target (the nodes). Hence, the connections radiating outwards from a node represent the contagion responses that that specific node induced in other nodes (i.e., the contagion induction influence). The inward connections received by a node correspond to the stimuli that such node received and responded to (sensitivity to contagion).

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Supporting material - Tables

Table S1

Group composition of bonobos housed at La Vallée des Singes

Subject	Sex	Age	Mother	Father
Daniela*	F	53	Margrit	Camillo
Ukela*	F	36	Natalie	Bono
Ulindi*	F	28	Natalie	Bono
Diwani*	M	25	Daniela	Masikini
David*	M	20	Daniela	Kirembo
Khaya*	F	20	Banya	Keke
Lingala	F	18	Jill	Mwindu
Lucy*	F	18	Lorel	Bosondjo
Kelele*	M	17	Salonga	Ludwig
Yahimba*	F	12	Kumbuka	Zamba
Loto*	M	12	Ulindi	Kumo
Moko*	M	9	Ukela	David
Khalessi*	F	9	Khaya	David
Yuli*	F	7	Lucy	Kelele
Swahili*	F	7	Lingala	Unknown
Lokoro*	M	6	Ulindi	Unknown
Kymia	F	4	Ukela	Unknown
Yago	M	0	Yahimba	Unknown

* Indicates individuals that were included in the yawn analysis

Table S2

Description of the behaviors considered for the present study. The behavioral items were extracted from the ethograms of Demuru and Palagi (2012), Kano (1980), Enomoto (1990) and de Waal (1988).

AFFILIATIVE BEHAVIOR	**DESCRIPTION**
Grooming	An individual clean another one's hair both with hands and/or mouth
Reciprocal Grooming	Two individuals grooming each other
Sit in Contact	Two or more individuals are sitting in reciprocal contact

Social Play	Two or more individuals play together. The most common forms of social play are the “rough and tumble”, that can include, for example, play slaps, play bites, play pushes etc., and the “play run”, where one subject chases another one
AGGRESSIVE BEHAVIOR	**DESCRIPTION**
Avoid	When an individual avoids interacting with another one, or when it changes its moving direction or goes far away from the latter
Bare Teeth	Facial expression of fear where all teeth are exposed; usually associated with screaming
Fleeing	An escape effectuated in an aggressive context
Screaming	A scream vocalization of fear
Urinate	An individual urinates for fear in an aggressive context
Defecation	An individual defecates for fear in an aggressive context
Aggressive Bite	An individual bites another one
Aggressive Brusque Rush	An individual jumps on another one
Aggressive Crouching	A crouching position assumed by an individual who is receiving an aggression, it is displayed to protect itself from the aggressor’s hits
Aggressive Push	An individual push another one by hands
Aggressive Pull	An individual pulls another one by hands
Aggressive Slap	An individual slaps another one by hands
Aggressive Stamping	An individual jumps on another one with feet together
Charging Display	It is composed of a series of behaviors (piloerection, run, facial expression, branch dragging, harm swinging, etc.) which generally are displayed by males to threaten other individuals, or to assess their dominance. In bonobo is common even within females
Chase	An individual chases another one

Kick	An individual kicks another one
Food Force Claim	Two individuals get close to food and one of them win in taking it

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Supporting material – Video

Video S1

Yawn contagion between Yuli (female of 7 years old) and Khalessi (female of 9 years old). Yuli emits the yawn at 11:53 a.m. and Khalessi responds after 6s. Khalessi is sitting within 1m from Yuli and can see the triggering yawn.

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CHAPTER 5

Yawning Is More Contagious in Pregnant Than Nulliparous Women - Naturalistic and Experimental Evidence

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Yawning is More Contagious to Pregnant Than Nulliparous Women: Naturalistic and Experimental Evidence

Ivan Norscia[1], Lucia Agostini[1], Alessia Moroni[1], Marta Caselli[1], Margherita Micheletti-Cremasco[1], Concetta Vardé[2], Elisabetta Palagi[3]

[1]Department of Life Sciences and Systems Biology, University of Torino, Italy

[2]Obstetrics and Gynecology Clinic (Pinerolo, Turin, Italy)

[3]Department of Biology, Unit of Ethology, University of Pisa, Italy

Corrisponding author e-mail: ivan.norscia@unito.it

Abstract

Contrary to spontaneous yawning, which is widespread in vertebrates and probably evolutionary ancient, contagious yawning - yawning triggered by others' yawns - is considered an evolutionarily recent phenomenon, found in species characterized by complex sociality. Whether the social asymmetry observed in the occurrence of contagious yawning is related to social and emotional attachment and may therefore reflect emotional contagion is a subject of debate. In this study we assessed whether yawn contagion was enhanced in pregnant women, a cohort of subjects who develop prenatal emotional attachment in preparation for parental care, via hormonal and neurobiological changes. We predicted that if yawn contagion underlies social and emotional attachment, pregnant women would be more likely to contagiously yawn than nonpregnant, nulliparous women of reproductive age. We gathered data in two different settings. In the experimental setting, 49 women were exposed to video stimuli of newborns either yawning or moving their mouth (control) and we video-recorded the women during repeated trials to measure their yawning response. In the naturalistic setting, 131 women were observed in a social environment and their yawning response was recorded. We tested the factors influencing the yawning response, including the reproductive status (pregnant *vs.* not pregnant). In both settings, yawn contagion occurred significantly more in pregnant than nonpregnant women. By showing that pregnant women were most likely to respond to others’ yawns, our results support the hypothesis that the social variation observed in yawn contagion may be influenced by emotional attachment and that yawning in highly social species might have been coopted for emotional contagion during evolution.

Keywords: Empathy evolution, Emotional contagion, Prenatal attachment, Maternal-fetal attachment

Introduction

Whereas spontaneous yawning is independent from the perception of others' yawns, contagious yawning occurs when the yawn emitted by a subject (trigger) acts as a releasing stimulus (*sensu* Tinbergen and Perdeck, 1951) and elicits yawning in another subject (responder) (Provine, 1989). Although morphological variants are present in yawns, especially in primates (e.g., chimpanzees, *Pan troglodytes*, Vick and Paukner, 2010; geladas, *Theropithecus gelada*, Palagi et al., 2009; Tonkean macaques, *Macaca tonkeana*, and Japanese macaque, *M. fuscata*, Zannella et al. 2017; humans, *Homo sapiens*, Provine, 1986, 2012), spontaneous yawning is probably a plesiomorphic (ancestral) trait because it has been recorded in a wide array of vertebrates (Baenninger, 1987). To the contrary, contagious yawning between conspecifics has been observed thus far in a relatively small number of species (Palagi et al., 2020) and may be an apomorphic trait, which appeared more recently in vertebrate evolution. With one exception (*Pongo pygmaeus*: van Berlo et al., 2020), the species exhibiting yawn contagion between conspecifics usually live in highly social groups: namely, all the extant hominine species (chimpanzees: Anderson et al., 2004; Campbell and de Waal, 2011; Campbell and Cox, 2019; bonobo, *Pan paniscus*: Demuru and Palagi, 2012; Tan et al., 2017; but see Amici et al., 2014 on a very small sample size; humans: Provine, 1986, 1989), two species of cercopithecines (geladas and Tonkean macaques: Palagi et al., 2009; Palagi and Norscia, 2019), non-primate mammals (lions, *Panthera leo*: Casetta et al., 2021; wolves, *Canis lupus lupus*: Romero et al., 2014; sheep, *Ovis aries*: Yonezawa et al., 2017; elephant seals, *Mirounga leonina*: Wojczulanis-Jakubas et al., 2019; domestic pigs, *Sus scrofa*: Norscia et al., 2021), and one social bird species (budgerigar, *Melopsittacus undulates*: Gallup et al., 2015).

One of the most remarkable aspects of intra-specific yawn contagion is that it shows social asymmetry in all the species where this aspect has been investigated (Palagi et al., 2009; Campbell and de Waal 2011, 2014; Norscia and Palagi, 2011; Demuru and Palagi, 2012; Massen et al., 2012; Romero et al., 2014). The yawning response is most likely or precisely triggered by yawns coming from individuals that are "socially relevant" to the potential responders, even though the communicative value of the triggering yawns (e.g., threat, tiredness) can vary. For example, in humans the yawning response is highest between familiar subjects (Norscia and Palagi, 2011). In chimpanzees, living in social groups characterized by male dominance, males seem to respond more when the triggering yawn comes from the dominant males (Massen et al., 2012), whereas in bonobos, living in groups with female dominance, females seem to be more effective in eliciting others' yawns (Demuru and Palagi, 2012). Indeed, bonobos and chimpanzees preferentially attend familiar subjects of the dominant sex (Lewis et al., 2021). In geladas, the female dyads -which are responsible for maintaining group cohesion-showed the most precise matching of different yawning types (Palagi et al., 2009).

The social attachment between individuals seems also to affect the rates of yawn contagion. In dogs (*Canis lupus familiaris*), the evidence of interspecific yawn contagion (dog/human) and its modulation is mixed (for review: Neilands et al., 2020; Palagi and Cordoni, 2020); in wolves, top rates of intraspecific yawn contagion were found between strongly bonded subjects (with bonding being measured by assessing the level of affinitive behavior; Romero et al., 2014). Adult chimpanzees (but not immature chimpanzees, Madsen and Persson, 2013) yawn more in response to the yawns of ingroup than outgroup members (Campbell and de Waal, 2011). Bonobos (in vivo but not when exposed to video stimuli; cf. Tan et al., 2017) show the highest yawning response between closely bonded individuals (Demuru and Palagi, 2012; Palagi et al., 2014). A similar situation occurs in geladas, with yawn contagion being greatest between individuals that affiliate the most (Palagi et al., 2009). In humans, yawn contagion is higher in kin and friends than in acquaintances and strangers (Norscia and Palagi, 2011; Norscia et al., 2016), and the familiarity bias remains when the yawns are heard but not seen (Norscia et al., 2020).

Based on neuroethological evidence, it has been hypothesized that in highly social species yawning may have been coopted during evolution for emotional contagion, a basic building block of empathy (de Waal and Preston, 2017; Palagi et al., 2020). However, at present, it is highly debated for both human and nonhuman animals whether the social asymmetry observed in yawn contagion depends on interindividual bonding, possibly reflecting emotional attachment -as postulated by the Emotional Bias Hypothesis (EBH)- and/or on other factors, such as attentional levels, social dominance, or as-yet undefined aspects of the social setting (Kapitány and Nielsen, 2017; Massen and Gallup, 2017; Adriaense et al., 2020; Palagi et al., 2020).

Emotional contagion and empathic processes are assumed to have evolved from mother-offspring bond (for review: Preston, 2013). Pregnant women are particularly suitable to investigate the link between yawn contagion and bonding because they undergo heavy psychological, physiological, and neurobiological changes leading to the development of maternal attachment and caregiving (Napso et al., 2018; Barba-Müller et al., 2019; Tichelman et al., 2019). These changes often alter body systems so that pregnant women perform and act differently (e.g., with respect to dietary choice, motor activity, sensitivity to emotional stimuli) than nonpregnant women in the general population (Crozier et al., 2009; Gradmark et al., 2011; Moya et al., 2014; Osório et al., 2018).

Although proposing different underlying mechanisms, definitions, and measures (Brandon et al., 2009), the psychological literature addressing attachment theory (originally introduced for the postpartum period; Bowlby, 1969) converges in indicating that mother-infant bonding starts long before birth, during pregnancy (Sedgmen et al., 2006; Sadeghi and Mazaheri, 2007; Ferrari et al., 2016; Salehi and Kohan, 2017). During gestation, women develop what Rubin (1975) called a sense of “we-ness,” later defined as prenatal attachment, the emotional and psychological bond between the mother and her unborn child (Brandon et al., 2009; Rossen et al., 2017). The mother-infant bonding quality developed in pregnancy is important because it is positively associated with the mother-infant bonding quality after birth (Tichelman et al., 2019).

Psychobiological changes during pregnancy, involving hormonal and maternal brain adaptations, occur in both human and nonhuman mammalian females to support the transition to parenthood (Lonstein et al., 2015; Kim, 2016). In women, the establishment of prenatal attachment is sustained by recent neurobiological evidence. Via magnetic resonance imaging (MRI), Hoekzema et al., (2017) found that during pregnancy women’s brains undergo dramatic, long-lasting changes in areas that significantly overlap with areas involved in the Theory of Mind (ToM) (i.e., anterior and posterior cortical midline and specific sections of the bilateral lateral prefrontal and temporal cortex; Hoekzema et al., 2017). ToM, among other aspects, is related to the ability to read others’ emotions (affective ToM; Abu-Akel and Shamay-Tsoory, 2011). Brain changes are also linked to the development of maternal attachment and can significantly predict the quality of future mother-infant attachment (Hoekzema et al., 2017).

Psychological and neurobiological changes are interconnected with the massive hormonal variations that occur in women during gestation (Glynn and Sandman, 2011; Barba-Müller et al., 2019). Changes in the so-called maternal brain (including areas especially involved in maternal caregiving) are mediated by glucocorticoids, prolactin, and oxytocin, whose levels increase across pregnancy (Prevost et al., 2014; Kim and Strathearn, 2016; Slattery and Hillerer, 2016; Napso et al., 2018). Moreover, prolactin -Growth Hormone (GH) family and neuroactive hormones, including melatonin and its precursor serotonin- prepare pregnant women to adequately care for their offspring by impacting on different physiological functions (Lévy, 2016; Napso et al., 2018). Oxytocin is the neuroactive hormone that is thought to play a major role in the development of maternal attachment and, more generally, social bonding in humans and other animals (Decety et al., 2016). Although contextual and inter-individual factors can mitigate or even reverse the effects of oxytocin (Beery, 2015; Olff et al., 2013), during pregnancy oxytocin is involved in the emergence of mother-infant emotional bonding and, in humans, also in the mental representations typical of such bonding (Feldman et al., 2007; Decety et al., 2016).

In summary, yawn contagion may be related to emotional attachment (as predicted by EBH), and pregnant women represent a cohort of subjects that is biologically and psychologically "equipped" for mother-infant emotional attachment (Brandon et al., 2009; Barba-Müller et al., 2019; Tichelman et al., 2019; Palagi et al., 2020). Hence, to check for further evidence of the association between yawn contagion and social attachment, possibly reflecting emotional attachment (de Waal and Preston, 2017), we focused on the yawning response in pregnant women. In particular, we predicted that if social asymmetry in contagious yawning is also driven by interindividual attachment -a proxy of emotional attachment- contagious yawning would occur at higher rates in pregnant compared to nulliparous women.

Material and Methods

The data for this study were collected from two distinct categories of women: pregnant women and nulliparous women -that is, women who were not pregnant and had no children. For the purpose of this study, we excluded from the nulliparous category women who had previously been pregnant because such experience is known to alter the maternal brain and the perception/recognition of infant and adult facial expressions (Kim, 2016; Hoekzema et al., 2017; Matsunaga et al., 2018).

Data were gathered in two different settings: the experimental setting, with the study subjects being isolated and exposed to video stimuli under controlled conditions (via trials), and the naturalistic setting, with the observational data collected on the study subjects in their environmental social context (no trials involved). The study subjects were different for the two data collection types (experimental and naturalistic). On the one hand, the experimental approach allowed the control or removal of certain variables (age, bond) but subjects were extrapolated from their social context. On the other hand, the naturalistic approach allowed the verification of the possible influence of pregnancy on the yawning response in ecological (but also more variable) conditions. Because either setting has advantages and drawbacks, we combined the experimental and naturalistic approach.

A yawn response can be considered to occur within 5 min after perceiving someone else's yawn (the trigger's yawn) (Provine, 1986), with a peak in the first minute (Provine, 2005; Palagi et al., 2014). However, in the fourth minute there is a higher probability of autocorrelation (meaning that the presence of a yawn performed by a subject at t0 increases the probability to have another yawn by the same subject at t(0+X), where X is the increasing unit of time; Kapitány and Nielsen, 2017). Therefore, we considered only responses that occurred within a three-minute time slot from the yawn emitted by the trigger (on video in the experimental condition and live in the naturalistic setting), in line with several previous works and to facilitate comparison (Anderson et al., 2004; Norscia and Palagi, 2011; Demuru and Palagi, 2012; Palagi et al., 2014; Norscia et al., 2016, 2020).

Experimental Setting

The video used for the experimental procedure (detailed in the next section) was composed of a black-and-white stimulus video and black-and-white neutral landscape videos. The stimulus video was built by joining 4-8 s clips showing two newborns (respectively 3 days and 3 months old, within the full breastfeeding period) while yawning (experimental condition) or moving their mouth (control condition) (Fig. 1).

Figure 1 Screenshots from the experimental video showing the control condition (top: babies moving their mouths) and the yawning condition (bottom)

The experimental and control clips of each newborn had the same duration and were extracted from the same videos within seconds, so they had the same framing, context, luminosity, contrast, and background. The total duration of the stimulus video (either yawning or mouth movements), including both babies, was 45 s. The clips were provided by the newborns' parents. Both parents signed a release document granting free use of the clips, including the possibility of showing and manipulating them for this research.

The videos with neutral landscapes were built from clips downloaded from a specialized website (pixabay.com). A beep sound added at the beginning of the video was downloaded (as.wav file) from freesound.org. Both the videos and the beep are available under Creative Commons CC0 license (Public Domain Dedication). Under this license, video and sound uploaders have waived their copyright and related or neighboring rights to the videos, which can be freely adapted and used without attributing the original author or source.

The full video (.avi) was obtained by merging the different videos into a single video (1820 × 720 px) in the following order: 20 s of neutral landscape video, the first stimulus video (45 s), 5 min of neutral landscape video, the second stimulus video (45 s), 3 min of neutral landscape video. The central period of 5 min of neutral landscape ensured that 3 + 2 min elapsed from the first to the second round of stimuli, in order to reduce the probability of autocorrelation.

The whole video was converted into black and white to remove any possible reference to the baby's gender (based on color of clothing or other cues). The video editing was carried out via the freeware Avidemux 2.7. Two versions of the full video were assembled, and both videos showed yawning and control stimuli; one video showed the yawning stimuli before the control (YC video), and the other showed the control stimuli before the yawn (CY video).

Experimental Setting and Study Subjects

A total of 292 experimental trials were carried out from June 2018 to January 2020, between 9:00 am and 7:00 pm. The trials with pregnant women were carried out at Dr. C. Vardé's Obstetrics and Gynecology Clinic (Pinerolo, Italy). Pregnant women (N=26, age range: 24-43 years old) participated in the trials on a voluntary basis during their monthly checkups at the clinic. Pregnancies ranged from 105 to 277 days (delayed delivery). Depending on their availability, the women underwent 1-6 trials (M=2.29; SD=1.21). Nulliparous women of reproductive age (N=23, age range: 24-40 years old) were tested at the Department of Life Sciences and Systems Biology (University of Turin, Italy) or in private houses. The sample size, with variably repeated measures, allows the use of Generalized Linear Mixed Models.

In all cases the trials were carried out in an isolated room to avoid any distraction or interference. The sample only included women who had slept at least 5 h, had no certified or declared disorders, and were not under pharmacological treatments that could alter the yawning rates (e.g., involving the use of psychoactive substances).

The subjects were white Italians, as inferred from physical traits and their last names. The newborns were unknown to all the tested subjects.

In compliance with the applicable regulations (Italian Legislative Decree no. 196/2003; EU General Data Protection Regulation 2016/679), women signed an informed consent in which they agreed to participate in the trials and granted permission (1) to be video-recorded during the experiment and (2) to have the video used for the purposes of this study. The exact purpose of the trials (recording yawning during pregnancy) was not revealed until the end of the study period when the women were told that the experiment was about the evaluation of attentional levels during pregnancy.

For each woman the trials were carried out by same experimenter (LA, AM, or MC). Right before the trial began, the woman was accompanied from the waiting room to a separate room and invited to sit on a chair in front of the screen, located at the height of the woman’s eyes. A camera had been previously located behind the screen at about 1.20 m distance, above the screen, so the face of the woman could be entirely recorded on video. The operator pressed the start button and the video started, preceded by 1 s blue screen with a beep sound. 20 s of neutral landscape clips were shown, while the operator left the room. After the first 20 s of neutral landscapes, when the woman was alone in the room, the first stimuli video started, marking the actual beginning of the trial. The woman watched the entire video, including stimuli and neutral sequences, lasting 9.21

min. The stimulus sequence (yawning/control or control/yawning) was randomized both within and across subjects (the neutral landscape clips were the same).

The face of the woman was recorded during the entire duration of the trial via a Canon Legria HFR36 to measure the number of yawns she exhibited while watching the experimental and the control video and in the following three minutes. The trials were carried out using a 15″ screen laptop (Core Processor i3 – i5, 2.40–3.7 GHz, 64 Bit, 4–8 GB RAM). The videos shown to the women occupied the entire screen.

Video analyses and data collection. Data were entered anonymously, by assigning an alphanumeric code to each woman. Videos were analyzed via the free software VLC 3.0.6 (©VideoLAN). For each trial the following pieces of information were included in the dataset: time, woman's code, reproductive status (nulliparous or pregnant), age, whether the woman yawned or not in the three minutes following the display of the first yawning or control stimulus, condition (yawning/control), the seconds the woman spent looking at the screen (attentional level), stimulus presentation sequence (yawning/control or control/yawning).

We categorized a yawn as such in these cases: (a) jaws open in a wide gape, deep inhalation, eye closing or narrowing (open yawns); (b) lip sealing, deep inhalation and at least one of the following patterns: nostril opening, eye narrowing, vacuum swallowing (nose yawns) (Provine, 2012; for vacuum swallowing: present study).

Data were extrapolated from the videos independently by MC based on the above categorization and recoded by IN and EP. The average Cohen's for yawn recognition was $\kappa=0.94$, and only yawns with 100% agreement were included in the analysis.

The levels of attention to the stimuli were overall excellent ($M_{yawning}=44.952$ s; SD=0.271; $M_{control}=44.794$ s; SD=0.978; $M_{pregnant}=44.864$ s; SD=0.733; $M_{nulliparous}=44.855$ s; SD=0.842).

Naturalistic Setting: Study Subjects and Data Collection

For the data collection in the naturalistic setting we also considered pregnant and nulliparous women (as defined above). Observational data on the pregnant women (N=81) were collected live (with no video) in the waiting rooms of the Department of Obstetrics and Gynecology of the Hospital of Pinerolo (Turin, Italy) and data on the nulliparous women (who were not pregnant and had no children; N=49) of reproductive age (in their twenties, thirties, or early forties) were collected at the Department of Life Sciences and Systems Biology (students during breaks, before and after classes) and in other settings (e.g., workplaces, social events) in 2019. These data also included observations of two pregnant women. Data were collected when conditions allowed unobstructed observation of all the individuals present, focusing on small, isolated groups, in absence of external perturbing events (e.g., strong noises, sudden interruptions by others entering the room). During data collection the identity of each subject was anonymously indicated via an alphanumeric code. The women included in the database were observed for at least 30 min and did not show repeated or abnormal displacement behavior.

All of the nulliparous women were known by at least one of the authors who collected the data (IN, EP, LA), their basic information was known, including their reproductive state. As a further confirmation, none of the nulliparous woman showed signs of pregnancy or delivered between the end of data collection and the time this article was written. The pregnant women were not known personally, but the basic information needed for this study was obtained through conversation with the data collector (LA). Data were collected from between around 9:00 am and 11:00 pm by using the all-occurrences sampling method (Altmann, 1974), with the women not knowing that they were being under observation and without any evident external source of disturbance. Notes were taken -unnoticed- on the mobile phone or on paper. The training on yawn identification was carried out by IN and EP on the videos collected from June to December 2018 in the experimental setting. Only open yawns were considered in the naturalistic setting.

When a subject yawned spontaneously (no faked yawn) in presence of at least one observer (potential responder), the following data were entered in the calculation sheet: time, yawner dummy coded identity (trigger), the dummy coded identity of the woman (potential responder) who could perceive the yawn (distance within 5 m), reproductive status of the potential responder (whether the woman was pregnant or nulliparous), social bond between trigger and potential responder (stranger or acquaintances), whether the woman yawned in the three minutes after the trigger's yawn (yawning response). We collected 308 yawning bouts in the presence of pregnant and/or nulliparous women.

Based on Norscia and Palagi (2011), the social bond was defined as follows: strangers=subjects who met for the first time; acquaintances=subjects who personally knew each other and whose relationship was based on a third external element -that is, work/university (colleagues), friends in common (friends of friends), patient-doctor relationship. Only the cases in which the bond was known to at least one of the authors were considered.

As explained above, we considered the yawning response as occurring within a three-minute time slot from the yawn emitted by the trigger. To reduce the possible autocorrelation effect during yawn trains (subsequent yawns occurring within 3 min following a triggering yawn), only the first yawn following the last trigger's yawn was recorded as response.

Statistical Elaboration

To check for possible differences in the two cohorts of women (pregnant and nulliparous), we ran the parametric t-test for two independent samples on age (normal distribution: Kolmogorov-Smirnov test, P=ns) and nonparametric Mann–Whitney tests for two independent samples (Siegel and Castellan, 1988) on experimental time and declared sleep hours (nonnormal distribution, Kolmogorov-Smirnov test, P<0.05). Montecarlo randomization (10,000 permutations) was applied for experimental time and sleep hours to account for pseudoreplication (same women repeated in different trials).

To analyze the data from the experimental trials we ran three GLMMs (Generalized Linear Mixed Model). A GLMM was run to verify what contextual factors could have an effect on the presence of yawning response (N=292 cases). The occurrence of a yawning response was entered as a dependent, binomial variable (coded as presence=1, absence=0). The following fixed factors were included in the full model: condition (factor variable: yawning video stimulus=1; control video stimulus=0), attention level (numeric variable: number of seconds the woman looked toward the video stimuli), video sequence (factorial: yawning/control=1, control/yawning=2), time period (factorial variable coded as follows: 09:01 am-12:30 pm =1; 12:30-16:00 pm =2; 16:00-19:30 pm =3) (Giganti and Zilli, 2011). The woman's dummy coded identity (potential responder) was entered as random factor.

Two additional GLMMs were ran to check which individual factors could influence the yawning response for either the yawning (N=146) or the control (N=146) condition. In both models, the occurrence of yawning response was entered as a dependent, binomial variable (coded as presence=1, absence=0). The fixed factors in the full model were age (numeric variable) and reproductive status (factorial variable: nulliparous=0; pregnant=1). The woman's dummy coded identity (potential responder) was entered as random factor.

A GLMM was also run to verify what factors could have an effect on the presence of yawning response in naturalistic conditions (N=308 cases). The occurrence of yawning response was entered as a dependent, binomial variable (coded as presence=1, absence=0). The fixed factors in the full model were (a) reproductive status (factorial variable: nulliparous=0; pregnant=1), (b) social bond linking trigger and potential responder (factorial variable: strangers=0; acquaintances=1), and (c) time period (factorial variable coded as follow: 1=09:00 am-12:30 pm; 2=12:30-16:00 pm; 3=16:00-19:30 pm; 4=19:30-23:00 pm; Giganti and Zilli, 2011). The variables bond and reproductive status were inversely correlated (Kendall's Tau-b = -0.794, P< 0.05) so they were included in the model as possibly having a divergent influence on the yawning response. The dummy coded identities of trigger and potential responder were entered as random factors owing to the variably repeated or unrepeated measures on the subjects.

We fitted the models in R (R Core Team, 2020; version 3.5.3) using the function lmer of the R-package *lme4* (Bates et al., 2015). We verified the significance of the full model in comparison to a null model that only included the random factors (Forstmeier and Schielzeth, 2011). We used a likelihood ratio test (Dobson, 2002) 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 model using the R-function "*drop1*" (Barr et al., 2013). Since the response variable was binary, we used a binomial error distribution (link function: logit).

Results

Experimental Setting

No significant difference was found between the two cohorts with respect to age distribution (t-test for independent samples, $N_{nulliparous}$=23; $N_{pregnant}$=26; t=1.728; *df*=47; P=0.091), experiment time (Mann–Whitney via Montecarlo randomization: $N_{nulliparous}$=69; $N_{pregnant}$=77; U=2531.00; P=0.617), and declared sleep hours (Mann–Whitney via Montecarlo randomization: $N_{nulliparous}$=69; $N_{pregnant}$=77; U=2274.00; P=0.123).

We ran a GLMM to check for the possible influence of contextual factors (condition: yawning video stimulus/control video stimulus, attention level, time slot and video sequence) on the yawning response. We found a significant difference between the full model fitted versus the null model (likelihood ratio test: $\chi^2=34.997$, *df*=5, P< 0.001). Therefore, we moved on with a *drop1* procedure. The GLMM indicated a significant effect of the condition (Table 1), with the yawning response being higher in the yawning than in the control video condition (Fig. 2). No significant main effect was found for the other variables.

Table 4 Results of the GLMM including the following fixed factors: condition (factor variable: yawning video stimulus=1; control video stimulus=0), attention level (numeric variable: number of seconds the woman looked toward the video stimuli), video sequence (factor variable: YC=1, CY=2), time period (factor variable: 09:00 am-12:30 pm=1; 12:30-16:00 pm=2; 16:00-19:30 pm=3). The identity of the potential responders (Responder) was included as random factor

	Estimate	**SE**	**Z-value**	**P**
(Intercept)[a]	-16.953	20.673	a	a
Condition (Y)[b,c]	2.275	0.489	4.655	**< .001**
Attention	0.305	0.459	0.664	.507
Time period (2)[b,c]	-0.976	0.631	-1.546	.122
Time period (3)[b,c]	-0.770	0.669	-1.151	.250
Sequence (2)[b,c]	0.195	0.410	0.474	.635

[a] Not shown as not having a meaningful interpretation.

[b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor.

[c] These predictors were dummy coded, with Condition (Y), Time period (2, 3), Sequence (2) being the reference categories.

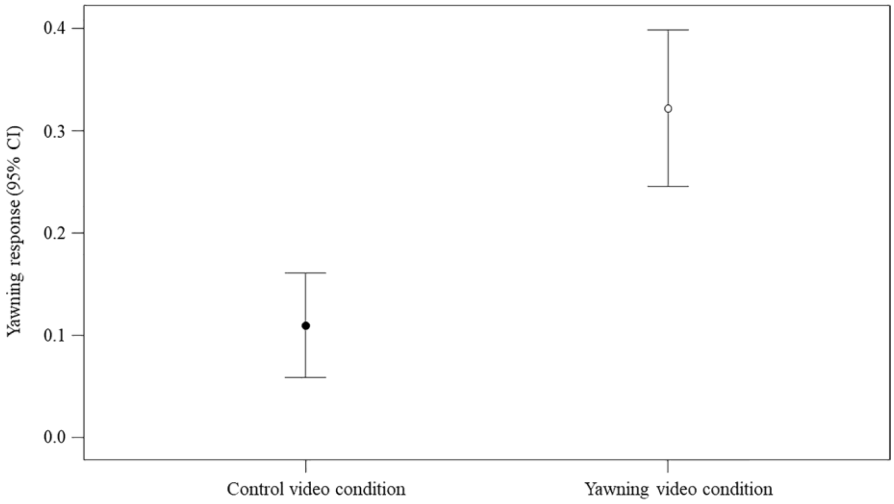

Figure 2 Effect of the type of the video condition (yawning/control) on the yawning response (experimental setting). Line plot showing the yawning response (Y axis) in the experimental setting as a function of the condition (yawning/control; X axis). The presence of a yawning response was significantly more likely in the yawning (M=0.3322; SE=0.039) than in the control (M=0.120; SE=0.026) condition (statistical results: Table 1), which confirms the presence of yawn contagion. Mean (circle) and 95% confidence interval (bars) are indicated

Subsequently, we ran two GLMMs on either yawning video condition data or control video condition data to test the possible effect of two individual factors (age; reproductive status: pregnant/nulliparous) on the yawning response. For the control video condition model, we found no difference between the full and the null models (likelihood ratio test: χ^2=0.391, *df*=2, P=0.822), with no predictor having a significant main effect on the response variable (age, P=0.653; reproductive status, P=0.596). Regarding the yawning video condition model, we found a significant difference between the full and the null models (likelihood ratio test: χ^2=6.140, *df*=2, P=0.046). Only reproductive status had a significant main effect on the response variable (Table 2), with pregnant women being more likely to respond to another's yawns than nulliparous women (Fig. 3).

Table 5 Results of the GLMM including the following fixed factors: age (numeric variable) and reproductive status (factor variable: nulliparous=0; pregnant=1); the identity of the potential responder was included as random factors

	Estimate	**SE**	**Z-value**	**P**
(Intercept)[a]	2.746	2.742	A	a
Reproductive status(1)[b,c]	1.684	0.813	2.071	**.038**
Age	-0.165	0.096	-1.715	.086

[a] Not shown as not having a meaningful interpretation.

[b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor.

[c] This predictor was dummy coded with "Reproductive status (1)" being the reference category

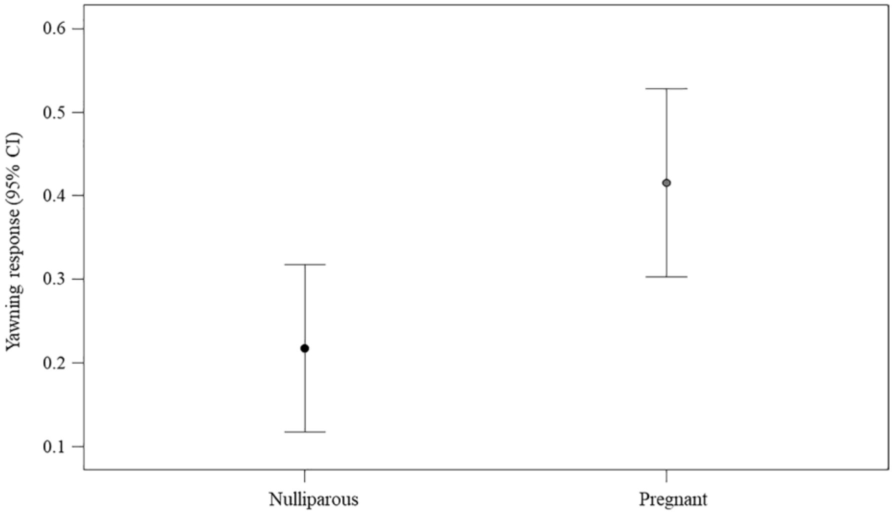

Figure 3 Effect of the reproductive status on the yawning response (experimental setting). Line plot showing the yawning response (Y axis) in the experimental setting as a function of the reproductive status of the woman potential responder (nulliparous/pregnant; X axis). The presence of a yawning response in the yawning video condition was significantly more likely (M=0.416; SE=0.057) in pregnant than in nulliparous (M=0.217; SE=0.050) women (Statistical results: Table 2). Mean (circle) and 95% confidence interval (bars) are indicated

Naturalistic Setting

We ran a GLMM to check for the possible influence of different factors (reproductive status: pregnant/nulliparous; social bond: strangers/acquaintances; time period) on the yawning response.

We found a significant difference between the full model fitted versus the null model (likelihood ratio test: χ^2=11.183, *df*=5, P=0.048). Therefore, we moved on with a *drop1* procedure. The GLMM indicated a significant effect of bond and reproductive status (Table 3), with the yawning response being higher in acquaintances than strangers (Fig. 4) and in pregnant more than nulliparous women (Fig. 5). No significant main effect was found for the time period.

Table 6 Results of the GLMM including the following fixed factors: reproductive status (factorial variable: nulliparous=0; pregnant=1), social bond linking trigger and potential responder (factorial variable: strangers=0; acquaintances=1), time period (factorial variable coded as follow: 09:00am-12:30 pm=1; 12:30-16:00 pm=2; 16:00-19:30 pm=3; 19:30-23:00 pm=4). The coded identity of trigger and potential responder were entered as random factors

	Estimate	**SE**	**Z-value**	**P**
(Intercept)[a]	-7.115	2.152	a	a
Reproductive status (1)[b,c]	4.650	1.745	2.664	**.008**
Bond (1)[b,c]	4.150	1.792	2.315	**.021**
Time period (2)[b,c]	1.009	0.869	1.161	.246
Time period (3)[b,c]	0.719	1.244	0.578	.563
Time period (4)[b,c]	-1.572	1.342	-1.172	.241

[a] Not shown as not having a meaningful interpretation.

[b] Estimate ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor.

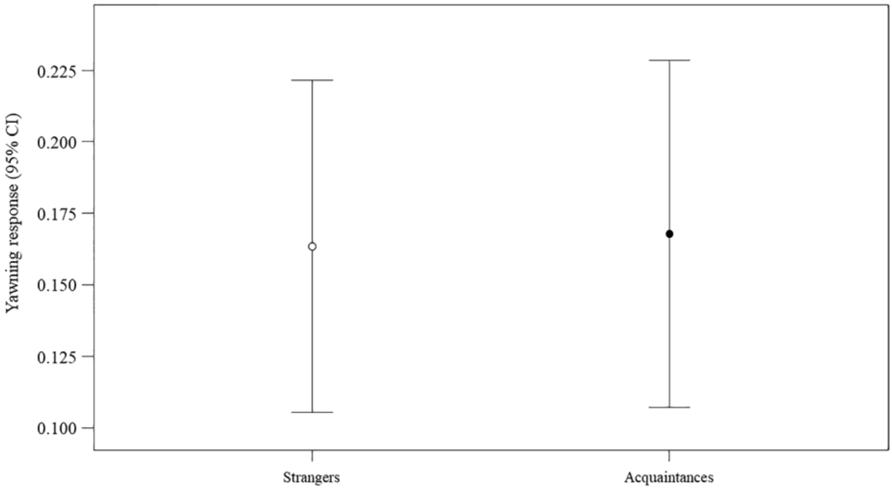

Figure 4 Effect of the social bond on the yawning response (naturalistic setting). Line plot showing the yawning response (Y axis) in the naturalistic setting as a function of social bond between trigger and potential responder (strangers/acquaintances; X axis). The presence of a yawning response was significantly more likely between acquaintances (Mean±SE: 0.168±0.031) than between strangers (M=0.164; SE=0.029) (statistical results: Table 3). Mean (circle) and 95% confidence interval (bars) are indicated

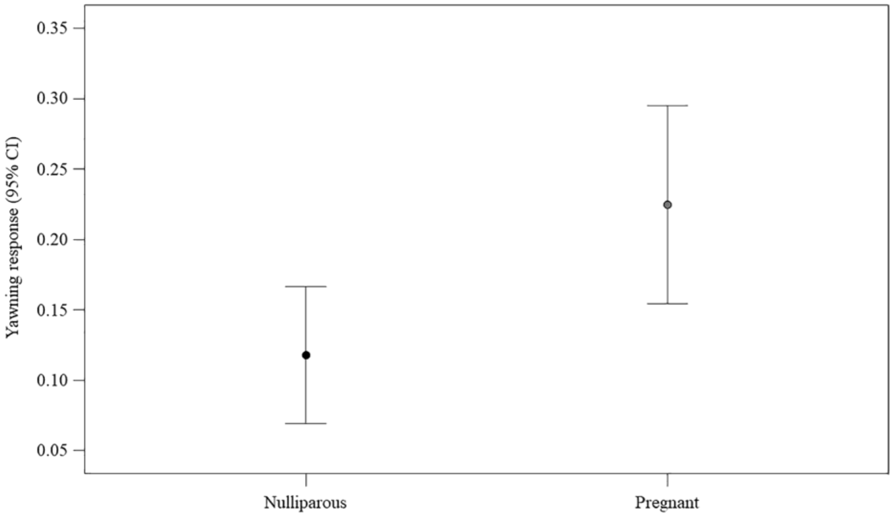

Figure 5 Effect of the reproductive status on the yawning response (naturalistic setting). Line plot showing the yawning response (Y axis) in the naturalistic setting as a function of the reproductive status of the woman potential responder (nulliparous/pregnant; X axis). The presence of a yawning response was significantly more likely in pregnant (M=0.225; SE=0.036) than in nulliparous (M=0.118; SE=0.025) women (statistical results: Table 3). Mean (circle) and 95% confidence interval (bars) are indicated

Discussion

The results from both the experimental and the naturalistic data converge in indicating that women's reproductive status had an effect on contagious yawning, which was more likely to occur in pregnant than in nulliparous women (here defined as women who were not pregnant and had no children). As a matter of fact, pregnant women were more likely to respond than nulliparous women to both video yawns of unknown infants in the experimental trials and live yawns from adults in the naturalistic setting (Tables 2 and 3; Figs. 3 and 5). This finding, presented for the first time with this study, provides support to the Emotional Bias Hypothesis (EBH) because yawn contagion was highest in the category of women characterized by enhanced social attachment predisposition, owing to the biological and psychological changes typical of the gestation period (Brandon et al., 2009; Barba-Müller et al., 2019; Tichelman et al., 2019).

Since yawn contagion has been found to vary across the day (Giganti and Zilli, 2011), we checked whether our yawning response sampling could be biased by the time periods during which the data were collected, depending on the availability of the study subjects. In neither setting did we find a significant effect (Tables 1 and 3), probably because the majority of the data was collected in the morning and in the afternoon (with little data collected at the very extremes of the day).

The use of a twofold approach, involving both experimental and naturalistic data collection, allowed the verification of the possible effect of different variables on yawn contagion. The results of the experimental trials show that the yawning response was significantly higher in the yawning than in the control video condition (Table 1; Fig. 2). This finding confirms that yawn contagion was present in the cohort of human subjects considered in this study (nulliparous and pregnant women) since it has been found in other segments of the population (Arnott et al., 2009; Provine, 1989, 2005).

Yawn contagion may be affected by selective, top-down attentional biases (Massen and Gallup, 2017), in addition to bottom-up, stimulus-driven attention (Attentional Bias Hypothesis, ABH; Palagi et al., 2020). Therefore, in the experimental setting we checked for selective attention to the *stimulus* and we found no significant influence of the time of attention to the stimulus source (video screen) on yawning (Table 1), which was high overall in both yawning and control video conditions, as well as in pregnant and nulliparous women. This finding reduces the probability that in our sample a selective attention bias may have accounted for the differences between stimulus (yawning/control) and reproductive status (pregnant/nulliparous) conditions. This is line with evidence indicating, directly or indirectly, that contagious yawning in humans may depend on bottom-up more than top-down selective attention (Norscia et al., 2020; for a review see Palagi et al., 2020). Age is another variable known to possibly affect yawn contagion rates (Bartholomew and Cirulli, 2014). In our case, in the experimental setting there was a nonsignificant trend of the influence of age in the yawning response, possibly because the women under study fell within the relatively short reproductive age.

In the naturalistic setting we could verify the effect of a social bond between the trigger and the potential responder on the yawning response. Although the bond was restricted to two categories (strangers and acquaintances) owing to data constraints, and despite showing an inverse correlation with reproductive status, the bond had a significant effect on yawn contagion, which was more likely between subjects who knew each other than between strangers. This finding is in agreement with previous literature showing that relationship quality has an influence on yawn contagion, whose likelihood increases as the strength of the social bond increases (from strangers to acquaintances, friends, and lastly to family members; Norscia and Palagi, 2011; Norscia et al., 2016). Norscia et al., (2020) found no difference between strangers and acquaintances when the yawns were heard but not seen, although friends and family responded at significantly higher rates than did those in the other categories. In the absence of the visual cue, it is probably more difficult for the potential responders to discern between subjects with whom they have reduced or no familiarity.

Importantly, our results from the experimental trials show that reproductive status (pregnant/nulliparous) had a significant effect on the yawning response in the yawning video condition but not in the control video condition (cf. Tables 2 and 3). Therefore, only yawning resulting from contagion -and not spontaneous yawning- was affected by pregnancy in our sample. Historical accounts report an increase of spontaneous yawning in the case of certain diseases (e.g., puerperal fever or hemorrhage; Walusinski, 2010), and excessive yawning has indeed been indicated as a possible marker of disease in humans (Thompson and Simonsen, 2015). Progesterone increases daytime drowsiness and sleeping time (Won, 2015) and so it may increase spontaneous yawning rate during pregnancy. In this respect, we cannot exclude that the yawning stimulus might have preferentially primed the yawning motor response in pregnant women also because they experienced increased fatigue (despite showing similar levels of sleep to those of nulliparous women). An investigation on how spontaneous rates vary within subjects across pregnancy, possibly in relation to fatigue and tiredness, and how contagious yawning varies depending on the stimulus (e.g., babies/adults) -with hormonal and neurobiological correlates- could better clarify the above issues.

Overall, the different yawning response of pregnant women relative to women with no children can fall within the broad range of the behavioral changes that start occurring during pregnancy, such as motor activity and dietary choice variations (Crozier et al., 2009; Gradmark et al., 2011). Compared with childless women, pregnant women show increased sensitivity to emotional signals and facial expressions. For example, pregnant women were found to perceive infant cries in more differentiated ways than women with no offspring (Bleichfeld and Moely, 1984; Yoshiaki, 1985). As gestation progresses, pregnant women also show enhanced ability to encode and process emotional faces, especially related to distress (an emotional state; Keltner et al., 2019) as an evolutionary adaptation to motherhood, which requires hypersensitivity to emotional threat signals and contagion (Pearson et al., 2009; Osório et al., 2018). Our results fit with this scenario because they indicate enhanced responsiveness of pregnant women to yawning, which has been linked (with various degrees of evidence) to anxiety and distress in human and nonhuman primates (from lemurs to apes: e.g., Baker and Aureli, 1997; Thompson and Bishop, 2012; Coleman and Pierre, 2014; Leone et al., 2014; Thompson, 2014, 2017; Zannella et al., 2015; Palagi et al., 2019). Thompson (2014) has posited that cortisol (involved in the stress response) may be involved in yawn contagion, at least under certain situations. Another hypothesis, not mutually exclusive to the cortisol hypothesis, may be that yawn contagion is, to a certain extent, under the influence of oxytocin, considering that enhanced emotional recognition is one of the effects of oxytocin, whose levels largely increase during pregnancy (Domes et al., 2007; Preston, 2013). In particular, oxytocin appears to increase the accuracy of the recognition of faces displaying angry and happy emotions, especially in women (Yue et al., 2018). Mariscal et al., (2019) found that yawn contagion in autism spectrum disorder (ASD) children was positively related to the blood concentration of oxytocin. The possible relationship between oxytocin and yawn contagion is supported by evidence that yawn contagion in humans follows the empathic gradient (*sensu* Preston and de Waal, 2002), being highest between closely bonded subjects (Norscia and Palagi, 2011; Norscia et al., 2020). Some features typical of mother-infant attachment, such as social recognition, bonding, and affiliation, are maintained in adulthood and promoted by oxytocin, which has been found to increase trust (Kosfeld et al., 2005), generosity (Zak et al., 2007), altruism (de Dreu et al., 2010), and both cognitive and affective empathy (Rodrigues et al., 2009; Shamay-Tsoory et al., 2013; Smith et al., 2014; Uzefovsky et al., 2015). One of the future steps is to evaluate the possible covariation between oxytocin and yawn contagion in both pregnant and nulliparous women. Beyond incorporating hormones, further studies could involve postmenopausal versus pregnant women and check how mothers react when they see their own fetus yawning on the echograph screen.

The possible connection between yawn contagion and increased social and emotional bonding is also suggested by the fact that some of the areas that seem to be involved in yawn contagion (such as the ventromedial-prefrontal cortex, superior temporal sulcus, amygdala, insula, posterior cingulate, and precuneus; Platek et al., 2005; Schürmann et al., 2005; Nahab et al., 2009) are also involved in mother-infant care, in mother’s enhanced sensitivity to the baby, and maternal brain changes occurring during pregnancy (Preston, 2013; Kikuchi and Noriuchi, 2015; Rifkin-Graboi et al., 2015; Hoekzema et al., 2017; Barba-Müller et al., 2019).

In summary, by showing increased occurrence of yawn contagion in pregnant women - a cohort of subjects that is specifically "programmed" to recognize and respond to others' emotions - this study provides support for the hypothesis that yawn contagion may, at least under certain circumstances, underlie emotional contagion (EBH; Palagi et al., 2020). This process is considered by some scholars a basic form of empathy and occurs when an emotion is transferred from one individual to another, possibly via a motor perception–action mechanism, involving the matching of facial expressions and the resonance of the emotions that underlie such expressions (de Waal and Preston, 2017).

The perception-action and the offspring care model both predict that subjects can preferentially attend the stimuli coming from closely bonded others, particularly caregiving individuals such as pregnant women toward babies (Preston and de Waal, 2002; Preston, 2013). Visual, top-down attention has limited effect on yawn contagion and does not follow a consistent familiarity trend in hominines because other factors, such as dominance, can come into play (Norscia et al., 2020; Palagi et al., 2020; Lewis et al., 2021). Hence, a possible bonding hypothesis between EBH and ABH is that yawn contagion can be influenced by emotional bonding and attention, mainly directed through bottom-up mechanisms.

Importantly, not all contagious yawning is triggered by emotional resonance, and that is not the point in question here. Contagious yawning also occurs between strangers (Norscia and Palagi, 2011), and some people are consistently not susceptible to others' yawns (Provine, 1986, 1989; Platek et al., 2003; Bartholomew and Cirulli, 2014). Contagious yawning is a form of yawning and -as such- can be related to non-emotional, individual and/or environmental factors, such as time of the day (Giganti and Zilli, 2011), age (Bartholomew and Cirulli, 2014), and possibly temperature (Gallup and Eldakar, 2011). The perception-action mechanism itself is based on a theory in motor control that assumes that our physical motor acts are primed in the brain by observation of those in others, even if they do not bear emotional cues (Preston and de Waal, 2002). Thus, contagious yawning can also be a non-emotional motoric response. The pivot around which this study revolves is the possible mechanism leading to the social variations observed in the occurrence of contagious yawning. Although still under debate (Massen and Gallup, 2017; Adriaense et al., 2020), various physiological, neuroethological, and psychological studies sustain the possible connection between the social asymmetry of yawn contagion and emotional bonding. Some of the brain areas that appear to be involved in yawn contagion (Platek et al., 2005; Schürmann et al., 2005; Nahab et al., 2009) seem to overlap with those involved in emotional processing of internal and external stimuli and empathy (Palagi et al., 2020) and -importantly- with the maternal brain (Kikuchi and Noriuchi, 2015; Rifkin-Graboi, et al. 2015; Hoekzema et al., 2017; Barba-Müller et al., 2019;). Highest levels of yawn contagion are associated with increased oxytocin levels (i.e., ASD children; Mariscal et al., 2019), enhanced social bonding (i.e., between friends and family; Norscia and Palagi ,2011), and maternal prenatal bonding (i.e., in pregnant women; present study). Lower yawn contagion rates in association with levels of autistic traits were found to be related to attentive rather than background emotional empathy deficits (Helt et al., 2021). Finally, another study found that subjects who yawned in response to observing others' yawns exhibited significantly higher empathy scores (Franzen et al., 2018).

Hence, although we cannot discard the possibility that other priming and motor mechanisms may also underlie the social asymmetry of yawn contagion, the hypothesis that this behavior has been coopted during evolution for emotional contagion still stands and gains further support.

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Data Availability

The raw data associated with this research are available in a Google Drive folder: https://drive.google.com/drive/folders/1O4MFD_nn-He0wtwyjozoi7JxxuP-WB8X?usp=sharing

Declarations

Ethical Statement - This ethological research project, completely noninvasive, is in compliance with the most recent privacy regulation (EU Regulation 2016/679; Italian DL 101/2018) was approved by the Bio-ethics Committee of the University of Torino (permit n. 195246).

Consent to Participate - In compliance with the national and European regulation (Italian Legislative Decree no. 196/2003; EU General Data Protection Regulation 2016/679), women were recruited after signing an informed consent through which they agreed to participate in the trials and granted permission

(a) to be video-recorded during the experiment and (b) to have the video used for the purposes of this study.

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GENERAL CONCLUSIONS

I would like to start my conclusion section by pointing out that the difficulties related to the COVID pandemics made it impossible to follow the original research plan so – based on the available data and what data collection was feasible or not - I redirected the focus of my research on different aspects more directly and indirectly to socio-emotional communication in different species. The most general objective of my PhD research was preserved though, as I showed how socio-emotional communication – in its multifaceted aspects - may occur in primates, spanning monkeys, apes and humans. Of course, as a preliminary study (chapter 1) I conducted a survey of the wild population of geladas to recognize at least part of the individuals and identify the best environmental conditions in which they could more freely express their behavioral repertoire (pasture rather than crop). I carried out the preliminary study in the light of the investigation to come on socio-emotional communication in this monkey species. This investigation was interrupted by the outburst – in 2020 - of the civil war in Ethiopia and the COVID pandemics. Despite this situation, I had enough data to at least investigate two aspects of socio-emotional communication in geladas, involving immature subjects. I started at a more ‘macroscopic’ level by assessing how infants can participate in shaping the socio-emotional relations in a monkey species, the geladas (chapter 2). Then, based on Preston and de Waal’s model (2002) of empathy I investigated more in-depth what factors could influence emotional communication via facial expressions. Both rapid facial mimicry and yawn contagion are as a matter of fact related to emotional contagion via the Preston and de Waal's Perception-Action Mechanism (2002), explained in the introduction of this thesis. In geladas I considered facial expressions that are commonly associated with positive affect (play face and its mimicry; chapter 3). I then considered facial expressions of neutral valence (yawning measured in relaxed conditions) in great apes (bonobos; chapter 4) and humans (pregnant women; chapter 5).

In geladas, infants play a role at two different levels: group level and population level. At group (OMU) and population levels, infants act as a sort of 'social bridge'. Within OMUs, infant handling enhances female grooming exchanges, that help them establishing, maintaining, reinforcing, and restoring social bonds (Dunbar, 1991) (chapter 2). Therefore, infant handling could be viewed as a female behavioral strategy allowing females belonging to the same OMU them to keep contact enhancing the probability to establish an emotional connection between them. From an evolutionary perspective, the fact that the phenomenon of infant handling could have a role in establishing an 'emotional bridge' between females, allows us to hypothesize that infant handling could represent the first step in the evolution of more complex forms of alloparental care - such as cooperative breeding - as it is shown in other primate species, such as

New World Monkeys and modern humans (Garber, 1997; Hrdy, 2009). As a whole, I confirmed the importance for gelada females, that live in female-bonded society, to establish and maintain strong social bonds via physical and emotional connection, which was increased by the presence of infants. Moreover, it could be interesting to evaluate if infant handling could influence both facial mimicry and yawn contagion between gelada females. At the population level, play units are the only positive contact between different OMUs (Dunbar and Dunbar, 1975). In this respect, my study found that the presence of context-independent signals such as Lip-Smacking could concur in establishing an affiliative mood where emotional communication is enhanced, and cooperative interactions are facilitated (chapter 3). Moreover, I confirmed that Rapid Facial Mimicry could be used not only to study the presence of motor mimicry (one aspect of the basal level of empathy in the Russian-Doll Model, Preston and de Wall, 2002), but also as an indicator of the presence of emotional contagion. In addition, I found that the frequency of play faces was higher in inter-OMU playful session than in intra-OMU ones - with the former being more unbalanced than the latter -, underling the importance to communicate the playful mood to the other players. Nevertheless, I found that the frequency of RFM was higher in intra-OMU playful sessions than in inter-OMU ones. This result is in contrast with what was I found on yawn contagion in adult geladas, in a co-authored study not included in this thesis. In that study (Gallo et al., 2021), my co-authors and I found that the yawning response was higher in males than in females and between different group rather than in the same group. As a whole, we can hypothesize that, on one hand, motor mimicry underlies socio-emotional communication between infants and that this phenomenon could play an essential role in maintaining the playful mood between players so that play can continue and function for motor training and for the assessment of the abilities of other individuals. On the other hand, yawn contagion may underlie emotional synchronization between adult males and could play a role in maintain high levels of tolerance between different OMU, which share the same home range and move from a place to another in coordinated way.

As regards socio-emotional communication in Hominini, studied via the phenomenon of yawn contagion, I confirmed the presence of yawn contagion in bonobos (chapter 4). Such finding – along with the results of previous studies - confirms that yawn contagion is likely present in all Hominini at the population level (chimpanzees: Anderson et al. 2004; Campbell & de Waal 2011; Campbell & Cox 2019; bonobos: Demuru & Palagi 2012; Tan et al. 2017; present study; but see: Amici et al. 2014; humans: Provine & Hamernik 1986) and therefore possibly in the last common ancestor. In particular, I found that in bonobos immature subjects are not concerned by yawn contagion, as it also occurs in geladas (Gallo et al., 2021), chimpanzees (Madsen et al., 2013) and humans (Anderson and Meno, 2003; Helt et al., 2010; Millen and Anderson, 2011; Cordoni et al., 2021). The most innovative result of my study concerns the role of reproductive females that elicited more responses from other females when showing sexual swelling. As reproductive females are central in bonobo society (Furuichi, 2011), my result supports the hypothesis that - as in other Hominini - the most influential sex can shape socio-emotional communication (in this case via yawn contagion). This study also introduces new elements on the factors that modulate yawn contagion, pointing towards the necessity of a meta-analytical approach combining observations across-groups and across-species (e.g., chimpanzees and humans). Future studies could verify if the mother-infant social attachment could have a role in determining the frequency of maternal yawning responses. It could be that, within mother-infant dyads, the mother could be more prone to respond to her infant's yawns compared to the yawns of another infant. By doing so, it would be possible to better understand the role played by the maternal hormones and the mother-infant attachment. This would be interesting from a cross-species comparative perspective also in the light of the results of my study on yawn contagion in pregnant women (chapter 5). The hormonal changes due to pregnancy, especially the increasing levels of oxytocin, might explain why pregnant women were more to contagiously respond to the yawns of a newborn than nulliparous women. Moreover, my results are in line with the hypothesis that yawn contagion may underlie emotional contagion, as mother-infant emotional attachment seems to have a role in increasing the rate of yawn contagion.

As a whole, with all the limitations related to the COVID pandemics and the civil war in Ethiopia, in my thesis I tried to follow the bottom-up approach to investigate the evolution of human socio-emotional communication by considering different primate. Thanks to this approach I was able to confirm the importance of socio-emotional communication in regulating inter-individual relationships – with a special focus on females and infants - in monkeys, great apes and humans. Such communication is fundamental in several contexts to build different types of social bonds, such as in mother-infant dyads (chapter 5) and in intra- (chapter 2) and inter-group dynamics (chapters 3 and 4). Moreover, the results of my doctoral thesis underline the importance of adopting a comparative approach on both closely and distantly related primate species, by applying the bottom-up perspective, to investigate the evolutionary pathways that may have led to the complex forms of socio-emotional communication found in humans.

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