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Berenty 2006: Recensement de *Propithecus verreauxi* et possibles signes de stress de la population

Introduction:

Ici nous présentons un étude sur un lémurien folivore, le *Propithecus verreauxi* (sifaka), qui vit dans la réserve de Berenty, au sud de Madagascar. Densités plus élevées de folivores dans des petits fragments se produisent dans les zones caractérisées par une concentration élevée de nourriture d'haute qualité ou dans des refuges moins perturbé. La asymétrie du rapport entre le quantité de males et de femelle, que dans le lémuriens est souvent favorable aux mâles, peut être exacerbée en condition de stress.

Prédictions:

Nous avons prédit que sifaka montrerait densités plus élevées dans les zones où la nourriture riche en protéines est plus abondante (prévision 1a), et dans le zones de refuge (prédiction de 1b). En raison de la compétition avec *Eulemur rufus* x *collaris* et *Lemur catta* et la diminution de la production alimentaire par les tamariniers, nous nous attendions à un *sex-ratio* biaisé vers les males (prédiction 2).

Résultats et Discussion

En Novembre-Décembre 2006, nous avons comptées et sexué 206 sifaka adultes/subadulte (49 groupes) au cours des promenades quotidiennes dans les différentes zones forestières (forêt secondaire d'Ankoba au nord, forêt galerie de Malaza, forêt dégradée et forêt épineuse, au sud). Le nombre de sifaka peut avoir diminué dans la forêt galerie (38 individus, 11 groupes) à se concentrer dans Ankoba (110 individus, 21 groupes). Ankoba est riche en protéines alimentaire (prévision 1a confirmé). Les sifakas sont proportionnellement plus concentré dans la zone épineuse, probablement utilisée comme refuge, que dans la forêt dégradé (prévision 1b confirmé). Le sex-ratio est extrêmement biaisé vers les males, peut-être due soit à forte densité de sifakas en Ankoba, ou à cause de la réduction de la disponibilité alimentaire en Malaza (prédiction 2 confirmé). La population de sifaka semble être dans une situation de stress: pour cela, c'est necessaire que les chercheurs reprennent les études démographiques sur les sifakas interrompues en Berenty dans les années 1980, au fin de préserver *in situ* une espèce qui est difficiles à protéger *ex situ*.

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Berenty 2006: Census of *Propithecus verreauxi* and Possible Evidence of Population Stress

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Abstract We provide a survey of a folivorous lemur, *Propithecus verreauxi* (sifaka), in the Berenty Reserve, southern Madagascar. Higher densities of folivores in small patches occur in either high-quality food areas or in less disturbed refugia. The skewness of sex ratio, which in lemurs is often male-biased, can be critically exacerbated in population stress. We predicted that sifaka would show higher densities in areas where protein-rich food is abundant (prediction 1a) and in refugium areas (prediction 1b). Owing to increased competition by brown and ring-tailed lemurs and decreased food production by tamarind trees, we expected an extremely male-biased sex ratio (prediction 2). In November–December 2006, we counted and sexed 206 adult/subadult sifaka (49 groups) during daily walks in different forest zones (Ankoba secondary forest, to the north, Malaza gallery/scrub areas, and spiny forest, to the south). Sifaka may have decreased in the gallery forest to concentrate in Ankoba (in a sort of out-of-Malaza). The area contains protein-rich food (prediction 1a confirmed). Sifaka are proportionally more concentrated in the spiny area than in the degraded scrub forest (prediction 1b confirmed). The sex ratio is extremely male biased, possibly due to either high sifaka density, in Ankoba, or food availability reduction, in Malaza (prediction 2 confirmed). The sifaka population seems to be under stress: researchers need to resume demographic studies, interrupted in Berenty in the mid-1980s, to preserve *in situ* a species that is difficult to protect *ex situ*.

Keywords drought · Indriidae · population survey · prosimian

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Introduction

Habitat fragmentation seriously threatens the survival of lemurs and other animal populations in Malagasy forests (Mittermeier *et al.* 2006; Ramanamanjato 2000). Population estimates are unavailable for many lemur species, and the absence of a clear understanding of the effects of habitat reduction on lemur populations, makes it impossible to implement effective conservation plans (Glessner and Britt 2005; Mittermeier *et al.* 2006). Density and abundance estimations are important tools to monitor lemur population conditions (Feistner and Schmid 1999; Lehman *et al.* 2006a). In fact, density data represent a valuable tool for management because they are used to derive population sizes, which in turn are valuable parameters for assessing population viability (Chiarello 2000; Shaffen 1981).

Besides density, sex ratio—the proportion of the individuals of each sex in a population—is also a key parameter to monitor population health, because it can dictate mate competition/choice and affect population growth rates (Emlen and Oring 1977; Ricklefs 1990). Deviations from an equal sex ratio are common in mammalian populations, and according to a complex framework of models and hypotheses, either adaptive mechanisms (social or parental control of sex ratio from conception onward: Nunn and Pereira 2000; Packer *et al.* 2000; Trivers and Willard 1973; van Schaik and van Noordwijk 1983) or nonadaptive ones, e.g., unpredictable environmental events leading to different mortality rates in the sexes (Bonefant *et al.* 2003; Kruuk *et al.* 1999), can shift primate sex ratios from 50/50. Consequently, the sex ratio of a population informs population viability.

We focus on the density and sex ratio of diurnal *Propithecus verreauxi verreauxi* (sifaka) in the Berenty Reserve, one of the last remaining gallery forest fragments of Madagascar (Jolly *et al.* 2006).

Propithecus must deal with strict energetic constraints related to energetically expensive locomotion (vertical leaping: Warren and Crompton 1997) and low-quality diet based on leaves (Norscia *et al.* 2006). Such a combination of features makes sifaka difficult to keep in captivity; consequently, protection plans need to be implemented *in situ*.

Propithecus occurs at greatly different densities in Malagasy forests; top densities are registered for *P. verreauxi* in small riverine forests in the south and the west, possibly in relation to steep ecological gradients and disturbance by people (maximum density: 400–500 individuals/km^2 at Antserananomby; Sussman 1974).

At Berenty, Jolly (1966) and Richard and Dewar (1991) studied sifaka behavior and basic demography (spanning 6–36 groups) until the mid-1980s. We could find no datum on population demography for the years since then. Considering the ecological changes that have occurred in the reserve, e.g. progressive land drying, decrease of tamarind cover, and exceptional growth of brown lemur populations (Blumenfeld-Jones *et al.* 2006; Jolly *et al.* 2006), it was crucial to resume research on Berenty sifaka demography. Via an intensive comprehensive survey, we aimed to clarify the situation of the Berenty sifaka population by testing some hypotheses on density and sex ratio and by contrasting our results with previous reports.

Prediction 1

Habitat perturbation can produce different trends of density variation in primate populations, depending on their ability to cope with forest destruction. Various primate species occur at higher density in disturbed forests than in intact ones, possibly owing to their behavioral flexibility, e.g. in terms of home range and diet (Onderdonk and Chapman 2000; Tutin *et al.* 1997).

Lemur density varies with the type and level of habitat perturbation (Ganzhorn 1995; Smith *et al.* 1997). Lower densities are usually associated with heavy disturbance because disturbance can affect food availability, as observed in *Propithecus edwardsi* and *Varecia variegata variegata* (Lehman *et al.* 2006a), *Avahi meridionalis meridionalis* (Norscia 2008), and *Eulemur collaris* (Ralison *et al.* 2006). However, low levels of disturbance, e.g., logging, may have a positive effect on leaf chemistry and enhance forest productivity, by allowing light penetration into the canopy and through forest edges (Ganzhorn 1995, 1997). The density of edge tolerant species increases in case of moderate perturbation, e.g., *Propithecus edwardsi*; *Hapalemur griseus*, *Avahi laniger* (Lehman *et al.* 2006b) and for *Microcebus* sp., *Cheirogaleus medius*, *Phaner furcifer*, and *Propithecus verreauxi* (Ganzhorn 1995).

The density of folivores follows leaf quality, mainly linked to protein content (Chapman *et al.* 2002; Ganzhorn 1992). Moreover, the lemurs that include a substantial amount of leaves in their diet, like *Propithecus verreauxi verreauxi*, should be more resilient than frugivores are to habitat disturbance. In fact, when the surrounding area is highly perturbed, folivores can potentially constrict their home range because leaves are more homogenously distributed in the forest than fruits are (Chiarello and de Melo 2001; Norscia 2008). Accordingly, one likely possibility is that higher densities in small areas can be the result of an artificial concentration of individuals in less disturbed refugia, e.g., like *Indri indri* (Glessner and Britt 2005).

In spite of its small area (200 ha), the Berenty Reserve is characterized by an environmental patchwork of 5 habitat zones, very different in terms of resource availability/distribution and habitat degradation (*cf.* Jolly *et al.* 2006; Simmen *et al.* 2003; Soma 2006).

Considering the aforementioned framework, we expect to find a higher density of sifaka either in areas characterized by protein-rich food (prediction 1a) or in the areas surrounded by a degraded matrix (prediction 1b).

Prediction 2

Unbalanced sex ratios (toward either males or females) occur in both birth and adult sex ratios of primate and nonprimate species (*cf* Allainé *et al.* 2000; Cockburn 1990; Packer *et al.* 2000; Pochron and Wright 2003; Pochron *et al.* 2004; Wright 1999). Regardless of the mechanisms, adaptive or nonadaptive, involved in sex ratio deviation from parity, Clutton-Brock and Iason (1986), Kruuk *et al.* (1999), and van Schaik and Noordwijk (1983) noted an unbalanced sex-ratio in association with population density increase or food quality/availability decrease. More specifically, a higher proportion of males occurs in several primate species in saturated habitats, possibly due to the fact that during population density peaks or food shortage, males, which are often the migrating sex, have better breeding opportunities by dispersing and entering other groups (*cf.* Perret 1990; Richard *et al.* 2002; Rudran Fernandez-Duque 2003; van Schaik and van Noordwijk 1983). Even if a male-biased sex ratio is common in lemurs (Kappeler 2000; Richard and Dewar 1991; Wright 1999), it is reasonable to expect that sex ratio unbalance can be exacerbated under negative ecological pressures.

In the past 2 decades, important environmental changes have occurred in Berenty, including the decrease of lemur staple food provided by tamarinds in the gallery forest (Blumenfeld-Jones *et al.* 2006) and the growth of the young secondary forest of Ankoba, containing protein-rich food for lemurs (Jolly *et al.* 2006).

Accordingly, we expect to find an extremely male-biased sex ratio in the *Propithecus verreauxi verreauxi* of Berenty.

Materials and Methods

Study Site

The Berenty Estate is on the Mandrare River in Southern Madagascar and contains *ca.* 1000 ha of protected forest (a remnant of 6000 ha of original forest): the forest is distributed in 3 separated reserves: Bealoka (a 100-ha area of gallery forest; S 23.36°, E 46.25°), Berenty (200 ha with different habitats; S 25.00°; E 46.30°), and Rapily (a spiny forest parcel, *ca.* 700 ha; S 25.00°, E 46.17°) (Jolly *et al.* 2002, 2006). The de Heaulme family established the reserves in consultation with local Tandroy clans, beginning in 1936 when they founded a sisal plantation in the area (Jolly 2004; Jolly *et al.* 2006).

We and 2 field assistants surveyed the sifaka in the Berenty Reserve from mid-November to the end of December 2006 (early wet season: Jolly *et al.* 2006).

Berenty Reserve comprises the northern section (Ankoba; S 24.99°; E 46.29°) and the southern section (Malaza, S 25.01°; E 46,31°). Ankoba is a 40-ha secondary forest 50–60 yr old, with canopy at 10–15 m and some emergent acacias (*Acacia rovumae*) to >20 m. It has an abundance of the exotic legume species *Pithecellobium dulce*, which represents a protein-rich food for lemurs (Jolly *et al.* 2006). Other introduced plant species grow in the area, e.g., *Azadirachta indica* and *Leucaena leucocephala*, together with a variety of tree and bush species that also occur in Malaza, e.g., *Celtis bifida*, *Capparis sepiaria*, *Ficus* spp., *Physena sessiliflora*, *Rinorea greveana*, *Tricalysia* sp., and the introduced *Cordia sinensis* (Norscia *et al.*, *unpub. data*; Simmen *et al.* 2006; Soma 2006).

According to Jolly *et al.* (2006, p. 36), "...the 100 ha of Malaza are subdivided into four habitat zones: front, gallery, scrub, and the spiny forest." Although spiny habitat is present in Malaza (at the southeast corner of the scrub area), proper spiny forest is located at the Southwest, over the bank called Red Hill.

The front part of Malaza western edge, including tourist buildings, contains a mixture of planted trees and tamarinds and usually hosts lemurs moving to and from the gallery forest (Jolly *et al.* 2006). Thus, we consider the front as part of the gallery forest area. The gallery forest, characterized by several arboreal species, is dominated by tamarinds (*Tamarindus indica*; Blumenfeld-Jones *et al.* 2006) and includes a transitional area (toward the most arid part of Malaza). The scrub area, characterized by isolated tamarinds and lower and succulent species, sharply grades uphill into the arid spiny forest, dominated by *Allauadia procera* (Didieraceae) and also contains other xerophytes and spiny bushes (Jolly *et al.* 2006).

Beyond Malaza, we included in our census the spiny forest parcel of about 10 ha: HAH Reserve Forestiere 2 (Fig. 1). The spiny forest continues with a further 38–40 ha beyond the road on de Heaulme land and connects to at least another 500 ha of more degraded spiny forest on Tandroy village owned land (Bedaro), which in turn

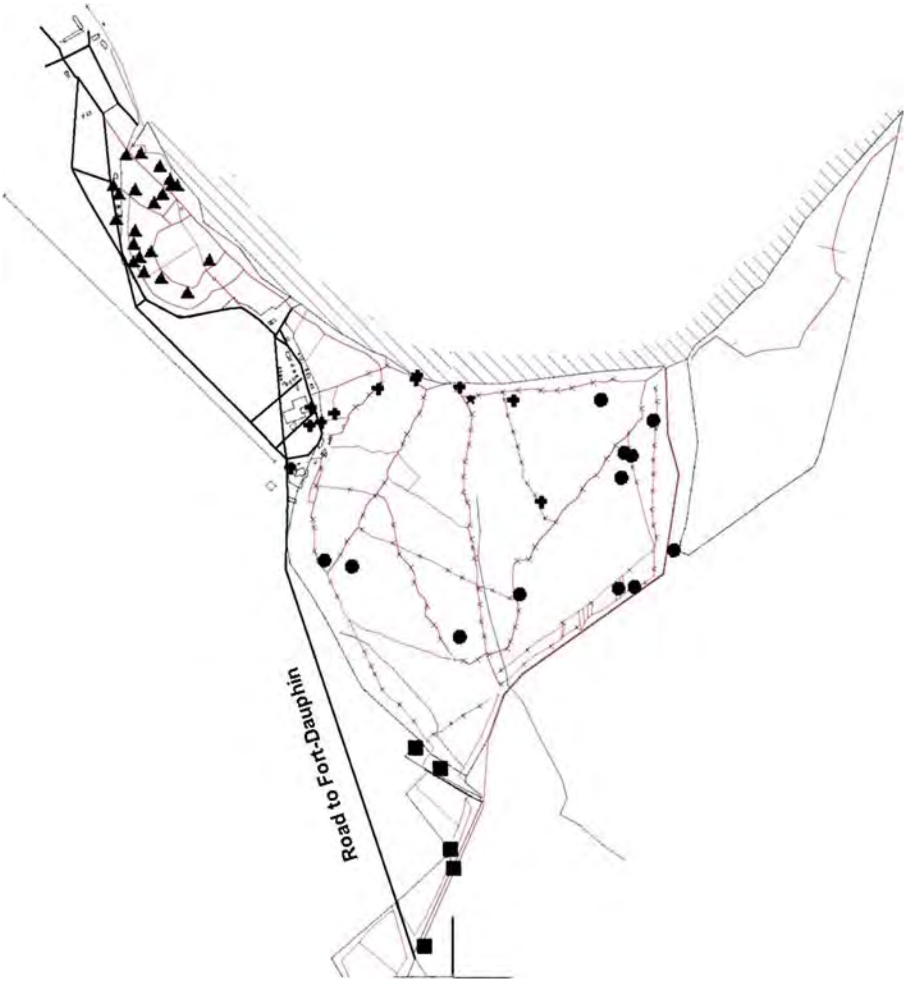


Fig. 1 Distribution of 49 groups counted in the Berenty Reserve in 2006: groups of Ankoba (triangles), Malaza gallery/transitional area (crosses), Malaza scrub area (dots), and spiny forest parcel (squares). The central part of the scrub forest appears to be characterized by absence of lemurs. (Map courtesy of G. Williams ©; scale 1:20,000).

connects to further parcels of spiny forest on de Heaulme land, some of them used by Tandroy people as a cemetery. We excluded it in our study.

Focal Species

In Berenty, *Propithecus verreauxi verreauxi* is sympatric with 5 other species of lemurs: *Lemur catta*, *Microcebus griseorufus*, *M. murinus*, *Lepilemur leucopus*, and an introduced hybrid population of *Eulemur fulvus rufus* × *E. collaris* (Soma 2006).

Propithecus verreauxi verreauxi is the smallest member of the genus, with an adult body mass of 3–4 kg. It occurs in the west, southwest, and extreme south of Madagascar (Tattersall 1982), and researchers have widely investigated them: Kirindy (Lewis 2005; Lewis and Kappeler 2005a, b), Beza-Mahafaly (Richard and Dewar 1991), Hazafotsy and Ampijoroa (Richard 1974), and Berenty (Jolly *et al.* 1982). The home range of *Propithecus verreauxi verreauxi* varies from 3 to >8 ha depending on the sites (*cf.* Jolly 1966; Norscia *et al.* 2006; Richard 1974, 1985), females are dominant over males and males are usually the dispersing sex (Richard *et al.* 2002).

In Berenty, the sifaka sex ratio shifted from a male bias to a female bias over 1964–80. In 1974 and 1975, when Jolly *et al.* (1982) counted >10 groups in 97 ha; Wilcoxon test: $n_{1974}=17$, ties=6, $T=4$, ns; $n_{1975}=16$, ties=7, $T=2$, ns) the number of males and females did not differ significantly (sex ratio: 0.54 in 1974, 0.57 in 1975). In other years, the sex ratio could be skewed by small sample size ($n \leq 10$ groups; e.g., in 1963–64 and 1970–71 sex ratio of 0.46–0.47; Jolly *et al.* 1982). Because sifaka live in groups of highly variable male-female composition, sex-ratio estimates derived from a small number of groups are particularly susceptible to sampling error (Richard and Dewar 1991).

Sifaka Census

Our census covered 157 ha and performed on consecutive days over 2 15-d periods. To perform a reliable count of sifaka groups and individuals, we used 2 different approaches in the different parts of the forest: Ankoba/Malaza gallery forest and Malaza scrub area/spiny forest parcel.

In Ankoba, sifaka distribution is extremely dense (Jolly *et al.* 2006), group size can be rather large (≤9 or 10 individuals), and it becomes very difficult to distinguish one group unit from another when the individuals of different groups spread out and mix with individuals of other groups during daily activities. In Beza-Mahafaly, Richard *et al.* (1991) reported that the individuals of one group, typically males, can visit other groups as a part of group transfer. We conducted our census in the premating period, which one can characterize by high intergroup mobility. In Beza-Mahafaly, Brockman (1999) reported that males can visit females of other groups when the mating period approaches. To be able to distinguish the different groups and determine whether we had already counted an individual or not, we performed the census during sifaka resting periods (1100 h–1430 h) when individuals of each group huddled together in a tree or adjacent trees. We used the same method in Malaza gallery forest.

In contrast to Ankoba and Malaza gallery forest, Malaza scrub area and the spiny forest are characterized by an open canopy (Jolly *et al.* 2006): here sifaka groups are more dispersed and it is possible to distinguish clearly one group unit from another. However, it is difficult to spot them resting in isolated trees in the scrub forest or hidden in the sparse bushy vegetation. Therefore, we conducted the census in the morning (0730 h–0930 h) when the sifaka were active and we could detect their presence from the trail more easily.

We performed the group/individual count via walking, at a speed of about 1 km/h, along preexisting trails and through forest paths chosen *ad hoc* to have visibility of ≥50 m right and left. To reduce the chances of either counting groups twice, in Ankoba, or missing groups of individuals, in Malaza scrub area and in the spiny forest, 2 field assistants repeated the count in each portion of forest we patrolled on 1 d on the subsequent day. We also repeated the census because on 2 occasions, in Ankoba, we observed the fusion of 2 groups on 1 or 2 close trees for resting (the groups split again at the beginning of the afternoon feeding session).

We recognized groups on the basis of size, presence of infants, and easily identifiable external characteristics of some individuals. We counted and sexed the sifaka of each group; Berenty sifaka are habituated to human presence and can be observed at close range, often <5 m. We established the position of each group via a global positioning system (Garmin, eTrex) and processed the data points collected via a geographical information system (ARCVIEW GIS, 3.0a) in combination with the Animal Movement Analysis Extension (Wronski and Apio 2005). The density is expressed as the number of individuals (abundance) per unit area (ha) (Ricklefs 1990).

For Berenty sifaka, the birth period is June–July (Jolly 1972). Sexing infant *Propithecus* <6 mo old from behavioral observations can be difficult owing to similar external morphology of the reproductive organs (Pochron *et al.* 2004). We sexed 11 infants of 23 because we could provide a reliable sex identification only for 6-month-old sifaka, born in June and recognizable for being larger than the youngest ones, when they were separate from their mother and we could observe them closely. To facilitate comparisons with earlier reports, we express the sex ratio for adults and infants as the proportion of females to males (Richard 1985; Richard and Dewar 1991).

Statistical Analysis

Owing to sample deviation from normality (Kolmogorov-Smirnov, $p<0.05$) or too small sample size ($n=5$–23 when considering the sifaka groups in different forest portions), we applied nonparametric tests (SPSS 12.0 and StatXact, Cytel Studio).

We could not test the differences between the number of males and females in the spiny forest due to the small sample size ($n=5$ groups, which is too small for both nonparametric and parametric tests; with $n<10$ one cannot test the sample for normality). Thus, we provide only descriptive information for this part of the forest.

We used the Kruskal-Wallis test (followed by Dunn test *post hoc*; Zar 1998) to analyze the differences in number of individuals/groups across the different parts of the forest (Ankoba, Malaza gallery, and transitional forest, Malaza scrub forest). We applied the Wilcoxon test for dependent pairwise comparisons between the number of males and females at group level. We selected $\alpha=0.05$ as the level of significance and we identified a trend for $0.05 \leq \alpha < 0.1$. All tests are 2-tailed. In figures we use single, double, and triple asterisks to indicate significant ($p<0.05$), highly significant ($p<0.01$), and extremely significant ($p<0.001$) differences, respectively. We refer to the exact values of probability per Mundry and Fischer (1998).

Results

The descriptive statistics on size and composition of the groups counted in Berenty are in Table 1. The distribution of sifaka groups in the different parts of the forest is in Fig. 1. Lemurs appear to be absent in the central part of the scrub forest.

Sifaka Density and Group Size

In Ankoba (40 ha) we counted 110 individuals belonging to 21 groups and estimated the density as 2.75 individuals/ha. However, the individuals clustered together in an area of only 10.61 ha, which increases the density there to 10.36 individuals/ha, at least during resting time. Overall, the density in Malaza (100 ha) was 0.96 individuals/ha. In particular, the density was 1.86 individuals/ha in the Malaza gallery-transitional zone (38 individuals distributed in 11 groups in an area of 20.41 ha), 0.41 individuals/ha in the Malaza scrub area (37 individuals belonging to 12 groups in 90.47 ha), and 1.91 individuals/ha in the spiny forest parcel (21 individuals belonging to 5 groups in 11 ha; 1 group comprised only 2 individuals).

There is a significant difference among the sizes of the 49 groups (1–10 individuals) in the 4 areas and particularly between Ankoba and Malaza scrub area (exact Kruskal-Wallis test: $n_{\text{ankoba}}=21$, $n_{\text{gallery}}=11$, $n_{\text{scrub}}=12$; $n_{\text{spiny}}=5$; df=3, $p<0.05$, $\chi2=9.17$; Dunn test; Ankoba/Malaza scrub areas: $Q=1.08$, $p<0.05$; other pairwise comparisons between the different areas: $0.25<Q<0.94$, ns; Fig. 2).

Sex Ratio

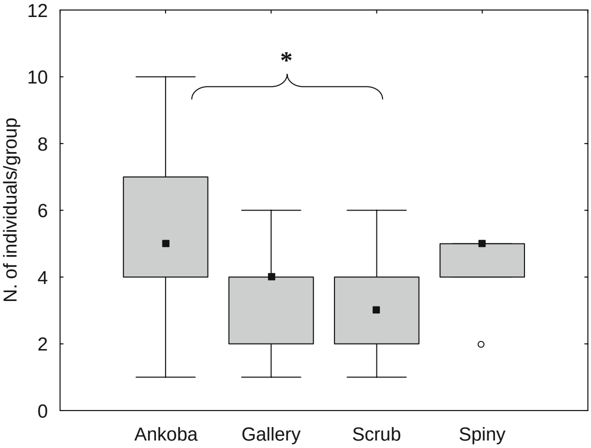
Overall, we counted 127 adult males and 79 adult females (adult sex ratio: 0.62).

Table 1 Descriptive statistics of the sifaka counted and sexed in Berenty

	Total number	Min/group	Max/group	Mean	STD
Groups	49	1	10	4.22	2.16
All individuals (infants and adults)	229	1	10	4.67	2.40
Adult males	127	0	7	2.59	1.62
Adult females	79	0	4	1.61	0.89
Infants	23	0	2	0.47	0.62

The table contains the total number of groups and individuals, number of adult males and females, and number of infants; minimum, maximum, and standard deviation (STD) of the number of individuals (of both sexes), males, females, and infants per group (valid $n=49$).

Fig. 2 Box plot graph representing group size variation in the 4 parts of the forest: Ankoba, Malaza gallery/transitional area, Malaza scrub forest, and Malaza spiny forest. The empty circle indicates an outlier point. The difference is significant between Ankoba and Malaza scrub area (single asterisk). Middle point (black square): median; box: 25–75%; whiskers: nonoutlier range.



Male sifaka significantly outnumbered females in the whole forest (Wilcoxon test: $n_{\text{gruppi}}=49$, ties=19, $T=5$, $p<0.001$; Fig. 3). When we considered Ankoba and Malaza separately, males also significantly outnumbered females (Wilcoxon test: $n_{\text{ankoba}}=21$, ties=7, $T=2$, $p<0.01$, Fig. 4; $n_{\text{malaza}}=28$, ties=12, $T=3$, $p<0.01$). However, when focusing the analysis on the groups in the 2 main areas of Malaza Forest the difference remains significant in Malaza gallery-transitional area (exact Wilcoxon test; $n_{\text{gallery}}=11$, ties=5, $T=0$, $p<0.05$; Fig. 4) but not in Malaza scrub area (Wilcoxon test: $n_{\text{scrub}}=12$, ties=5, $T=2$, ns; Fig. 4).

We could apply no test for the spiny forest due to small sample size ($n=5$ groups, including 12 males and 9 females). The sex ratio departs from parity in Ankoba, Malaza gallery-transitional area, Malaza scrub zone, and in the small part of spiny forest. In fact, the sex ratio values are 0.58, 0.57, 0.68, and 0.75, respectively.

Of the 49 groups, 17 included 1 infant and 3 included 2 infants, for a total of 23 infants. Of the infants for which we could identify the sex (11 of 23), the difference

Fig. 3 Box plot showing the difference between the numbers of adult males and adult females in the whole forest. The difference is extremely significant (triple asterisk). Black squares: median; box: 25–75%; whiskers: nonoutlier range.

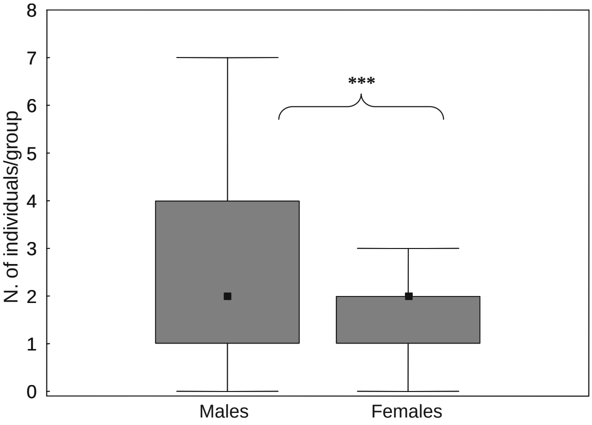
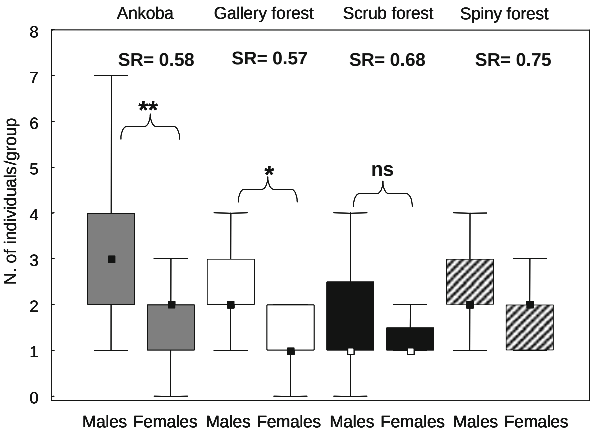


Fig. 4 Multiple box plot showing the difference between the number of adult males and adult females in Ankoba (highly significant: double asterisk), Malaza gallery/transitional area (significant: single asterisk), Malaza scrub area (nonsignificant, ns) and spiny forest area (we applied no test because of the small sample size).The sex ratio (SR) for each area is also indicated. Black square: median; box: 25–75%; whiskers: nonoutlier range.



between males and females (total infant males: 9; total infant females: 2) trended toward significance (exact Wilcoxon test; $n=9$, ties=1, $T=1$, $p=0.063$).

Considering data from previous studies, the divergence between the number of males and females of *Propithecus verreauxi verreauxi* is not significant over 1963–2006 (1963–85: Richard and Dewar 1991; 2006: present study; exact Wilcoxon test: $n=11$; ties=0; $T=4$; ns; Fig. 5).

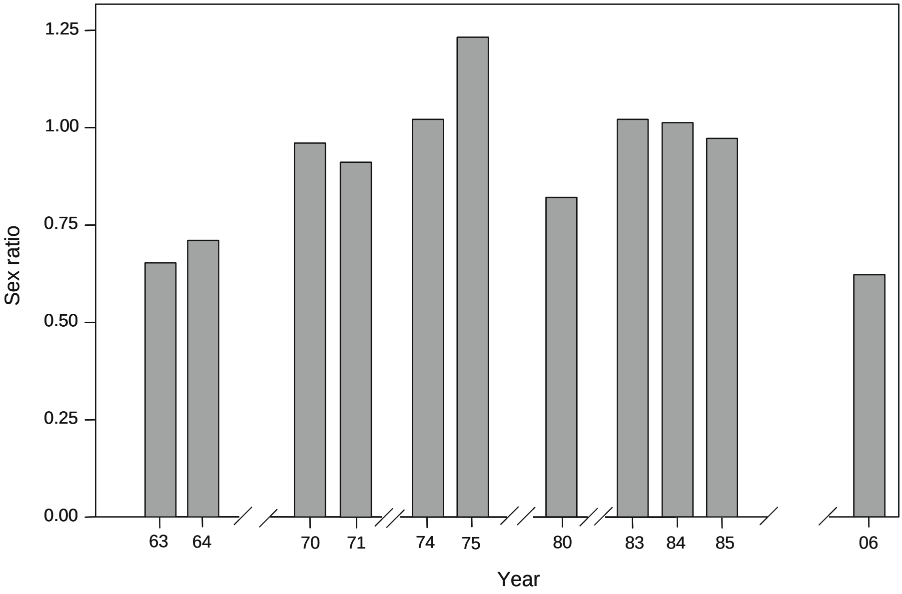


Fig. 5 Sex ratio variation in Berenty sifaka. Data from 1963 to 1985 refers to 9–36 counted groups and are taken from Jolly (1972), Richard (1974), Jolly *et al.* (1982), Richard (1985), Richard *et al.* (1991). To our knowledge, no published data are available for 1986–2005.

Discussion

Differences in Population Density

Our results suggest a tendency for sifaka to polarize toward the extremes of the fragment: in the northern part, Ankoba, and in the southern part, the spiny area. In fact, there was a higher density of sifaka in Ankoba (2.72–10.36 individuals/ha), where introduced species dominate the vegetation (Jolly *et al.* 2006). Moreover, sifaka were proportionally more concentrated in the tiny parcel of spiny forest than in the scrub zone (1.91 individuals/ha). Indeed, sifaka have shown a preference for plants originating in the spiny forest and for introduced plant species in Ankoba (Simmen *et al.* 2003).

The density peak in Ankoba is likely to be associated with the abundance of protein-rich food in the area (Jolly *et al.* 2006; prediction 1a confirmed). Being a recent secondary forest (Jolly *et al.* 2006), Ankoba may not have been as rich a habitat 20 yr ago as it is today.

In the spiny forest parcel, the density of sifaka appears higher than expected (1.91 individuals/ha), especially versus their density in the scrub area (0.41 individuals/ha). In 1999, Ferguson (*pers. com.*) estimated a low density (0.37 individuals/ha) in a relatively intact spiny forest a few km from Berenty. If we interpret the density of sifaka in the spiny forest parcel, which is small, narrow, and surrounded by a degraded matrix, as the result of the artificial concentration of groups in a small refugium area, i.e., spiny forest better than scrub area, we can consider prediction 1b confirmed. Such a phenomenon occurs in disturbed habitats (Britt *et al.* 2002), and the harsh conditions of the central scrub zone in Berenty may favor it. In fact, the degraded core of the scrub forest, where we counted individuals via the same criteria used in the other areas of the reserve, seems characterized by the lack of lemurs (Fig. 1).

Overall, the density of Berenty sifaka in 2006 was 1.32 individuals/ha, much less than the density O’Connor (1987) estimated 2 decades ago: 2.11 individuals/ha.

From a long-term perspective, and considering that much information on the evolution of sifaka populations in the last 20 yr is missing, we can only sketch a scenario to explain the current density distribution of sifaka in Berenty (Fig. 1). Such a scenario, which takes into account that the forest size and outline have not changed (Jolly, *pers. com.*), involves 2 main events: the dramatic decrease, over the past 30 yr, of the cover of tamarinds, which represent a key food resource for lemurs in Malaza gallery forest, possibly related to changes of water dynamics (Blumenfeld-Jones *et al.* 2006) and the concurrent exceptional increase of hybrid *Eulemur fulvus* × *E. collaris* starting with inadvertent introduction of a few individuals in 1975 to Berenty (Simmen *et al.* 2003).

Indeed, population growth of *Eulemur* (479 adult/subadult individuals in 2006; Razafindramanana, *pers. com.*) may have exacerbated interspecific competition over the keystone resource *Tamarindus indica*. Though competition over *Tamarindus* is higher between *Lemur catta* and *Eulemur* (Pinkus *et al.* 2006), tamarinds provide staple food not only for *L. catta* but for all 3 completely/partially diurnal lemur species at Berenty, at least in the dry period (Mertl-Milhollen *et al.* 2006; Simmen *et al.* 2003, 2006). Tamarinds are important for *Propithecus verreauxi* at other sites, where they eat leaves, seeds, flowers, and especially fruits: Kirindy CFPF (Norscia *et al.* 2006) and Beza-Mahafaly (Yamashita 2002). The situation may have led some of the Berenty sifaka to replace food items of *Tamarindus indica* (becoming less and less available or exploitable in Malaza) with alternative edible items provided by introduced plants. As a consequence, sifaka would move and settle in Ankoba, where species like *Pithecellobium dulce* that provide protein-rich food (Jolly *et al.* 2006) grow progressively and build the secondary forest. The fact that the population monitored in 10 ha of gallery forest, apparently increasing between 1963 and 1975, had decreased to the original level by 1980, might support the hypothesis of a partial population shift from the tamarind area to other parts of the forest (Jolly *et al.* 1982).

From a short-term perspective, the decrease of food availability in Malaza due to the 2004–2006 drought in southern Madagascar may have affected the condition of tamarinds, which are particularly sensitive to water availability (Blumenfeld-Jones *et al.* 2006). The food decrease in Malaza may have also resulted in a temporary increase in the number of sifaka in Ankoba. Indeed, in Malaza, sifaka, more than sympatric ring-tailed and brown lemurs, seem to rely on introduced species (Simmen *et al.* 2003). Accordingly, the Ankoba sifaka base their diet mainly on introduced species, which dominate the secondary forest, e.g., *Pithecellobium dulce* and *Azadirachta indica* account for *ca.* 50% of the diet (Norscia *et al.*, *unpub. data*).

Overall, the 3 aforementioned events may have contributed, in different ways, to promote the out-of-Malaza movement of sifaka.

Male-biased Sex Ratio

The population of Berenty sifaka is extremely unbalanced toward males (prediction 2 confirmed), which might not be surprising *per se*. In fact, lemurs (contra anthropoids) are characterized by the fact that adult males often outnumber adult females (Kappeler 2000; Richard and Dewar 1991; Wright 1999). Accordingly, a male-biased sex ratio occurs, at least in some years, in *Eulemur fulvus*, *Propithecus edwardsii* (Ostner and Kappeler 2004; Pochron and Wright 2003) and in *P. verreauxi verreauxi* of Kirindy CFPF (Ostner 2002; Lewis, *pers. com.*), Beza Mahafaly (Richard *et al.* 2002), and Berenty.

Over the years and across sites, sex ratios do not always depart significantly from parity (Richard and Dewar 1991). The divergence between the number of males and females of *Propithecus verreauxi verreauxi* has turned out not to be not significant over the span 1963–2006 (Fig. 5). In particular, males were not significantly more abundant than females either in 1974 or in 1975 (16–17 groups; Jolly *et al.* 1982). Such results are likely to be biased by sampling errors related to both sample size variation over time and census area (sifaka count always refers to a part of Malaza forest). Nevertheless, the sex ratio of Berenty *Propithecus verreauxi verreauxi* appears to be critically skewed in 2006. Indeed, the same year adult sex ratios of brown lemur and ring-tailed lemur populations were not particularly skewed in Berenty (brown lemurs: 247 adult/subadult males and 231 adult/subadult females; Razafindramanana, *pers. com.*; ring-tailed lemurs: 182 adult males, excluding solitary males, and 187 adult females in total; Ankoba: 70 adult males vs. 62 adult females; Malaza: 112 adult males vs. 125 adult females; Jolly, *pers. com.*). However, the sex ratio of *Lemur catta* has fluctuated (Jolly *et al.* 2002; Koyama *et al.* 2001).

The extreme male-biased sex ratio in Berenty sifaka (Fig. 3) may indicate that sifaka are particularly stressed, possibly because indriids are more vulnerable than lemurids are to resource limitation determined by increased competition or restricted food choice. Indeed, most lemurids choose higher energy foods than those of indriids (Godfrey *et al.* 2004), which combine energetically expensive locomotion with a low-quality diet (*cf.* Norscia and Borgognini-Tarli 2008; Warren and Crompton 1997). Moreover, while the diet of *Propithecus* spp. is varied but rather selective (*cf.* Norscia *et al.* 2006; Simmen *et al.* 2003; Yamashita 2002), *Eulemur* spp. and *Lemur catta* have very flexible diets, which can include invertebrates, in addition to fruits, leaves, and other plant parts (*cf.* Curtis 2003; Donati *et al.* 2007; Soma 2006; Tarnaud 2004). *Eulemur fulvus*, in particular, also eat small vertebrates and eggs (Nakamura 2004).

In Malaza, a contributing factor to the skewed sex ratio of sifaka (Fig. 4) may be food scarcity due to the decreased number of tamarind trees (possibly exacerbated by the drought of 2004–2006). Clark (1978) hypothesized that bush baby mothers would preferentially produce sons when food resources become scarce because males can potentially disperse far and find better territories with more abundant food.

In Ankoba, the highly significant difference in the number of males and females (Fig. 4) may be more related to the high density of sifaka in the area. Moreover, the fact that in the arid spiny and scrub areas the sex ratio is less unbalanced than in the other parts of the forests (Fig. 4) might be related to the baseline habituation of sifaka to harsh conditions in the spiny/scrub zone. Sifaka are a typical spiny forest species that inhabit them throughout southern Madagascar: they have also occurred in the gallery forest since its creation by the river several centuries ago, but the rich habitat is much rarer in the sifaka range (Jolly, *pers. com.*; *cf.* Jolly 1966; Tattersall).

The fact that 6-mo-old males were more numerous than females of the same age is not surprising. For example, in Beza-Mahafaly, more males than females were born in most years and young adult females died at higher rates than young adult males did (Richard *et al.* 2002). Moreover, droughts, which are part of the cyclical patterns in Madagascar (Wright 1999), are indeed a source of population stress, and they can increase mortality, especially of young females both in lemurs, e.g., *Lemur catta* (Gould *et al.* 1999, 2003), *Propithecus verreauxi* (Richard *et al.* 2002), and in other primates, e.g., tonkean macaques (Dittus 1988), chacma baboons (Hamilton 1985). In 1992, male sifaka in Beza-Mahafaly experienced less body mass loss than females did (Richard 2002). Thus, the drought in 2004–2006 may have contributed to push the sifaka sex ratio from parity, toward males.

Conclusions

Jolly *et al.* (2006) noted that if researchers had performed a population and habitat viability study at Berenty *ca.* 70 yr ago it would have seemed extremely unlikely that such a tiny fragment could survive the changes of later decades. Lemur densities in Berenty seem far from falling under the viable threshold proposed by Ganzhorn *et al.* (2003), who hypothesized that if lemur populations fall to *ca.* 40 adults they might be unable to survive for >40 yr in isolated forest fragments. Indeed, *Lemur* *catta* at Berenty may live at the highest density of all populations (Gould 2006), and *Eulemur fulvus* have experienced an incredible population expansion, from <10 individuals to >400, in the past 3 decades (Jolly, *pers. com.*). Sifaka density remains above the viability threshold, but the extremely male-biased sex ratio might indicate that the population is approaching carrying capacity in response of severe ecological changes.

The lack of complete censuses and demographic data in the last 20 yr does not allow us to state whether the sifaka population is experiencing a physiological/pathological expansion or contraction. However, based on the information from previous data, the Malaza population might have decreased over time, with part of it shifting to better areas, pushed or favored by long-term changes in critical ecological variables, e.g., decreasing tamarind population, increasing competition by *Eulemur fulvus*, and maturation of Ankoba secondary forest with high food quality (Blumenfeld-Jones *et al.* 2006; Jolly *et al.* 2006; Simmen *et al.* 2003).

It is crucially important to continue to monitor this population to understand the population dynamics, highlight possible risks, and implement conservation plans *ad hoc*.

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Rétablissement de la paix sur la cime des arbres: première preuve de la présence de réconciliation dans un prosimien en liberté.

Introduction

Le comportement de réconciliation est défini comme le premier contact affinitaire après d’un conflit entre les adversaires. Pendant que las réconciliation dans primates anthropoïdes a été largement étudiée, peu d'études ont étés effectués sur les prosimiens, et seulement en captivité. Contrairement aux anthropoïdes, les prosimiens malgaches sont caractérisés par une dominance des femelles sur les males, l’absence de dimorphisme sexuel et une reproduction saisonnière. Cependant, ils partagent avec anthropoïdes des caractéristiques tels que la coexistence dans sociétés cohésives, la philopatrie féminine et la capacité de se reconnaitre individuellement. La comparaison entre les prosimiens sociales et les anthropoïdes est cruciale pour comprendre l'évolution de la dynamique de réconciliation dans les primates, y inclus l’homme.

Nous présentons ici la première étude sur la réconciliation dans un prosimien, le Propithecus verreauxi (sifaka), dans la forêt de Berenty (sud de Madagascar). Nous avons examiné le comportement post-conflictuel à la lumière des coûts et des avantages potentiels des individus impliqués.

Resultats et Discussion

Nos résultats indiquent que *P. verreauxi* peut évaluer les risques et les avantages de se réunir a un adversaire après d’un conflit. Les victimes étaient les plus susceptibles d'interagir positivement avec l’agresseur, mais seulement après les conflits de faible intensité. En plus, seulement les conflicts qui se produisaient dehors du contexte alimentaire (quand les animaux n’étaient pas en train de manger) pouvaient être réconciliés. Ces résultats sont en accord avec le fait que *P. verreauxi* a une dominance sociale manifestée par un priorité de l'alimentation plus que dans le cadre des relations despotiques agressives. En accord avec l’hypothèse de las relations sociales valables (*valuable relationship hypothesis*), *P. verreauxi* étaient plus susceptibles de se réconcilier avec des partenaires valables: la réconciliation préférentiellement eu lieu entre subordonnés et individus d’haut rang et entre les animaux partageant de bonnes relations (expresses par des niveaux élevés de comportements affinitaires). À court terme, la réconciliation dans *P. verreauxi* semble avoir un rôle important dans la réduction de la probabilité de nouvelles attaques de l'agresseur.

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Peacemaking on treetops: first evidence of reconciliation from a wild prosimian (*Propithecus verreauxi*)

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Reconciliation is defined as the first postconflict affinitive contact between former opponents. While reconciliation in anthropoid primates has been widely investigated, few studies have focused on postconflict mechanisms in prosimians, and only in captivity. Unlike anthropoids, Malagasy prosimians show female dominance, lack of sexual dimorphism and seasonal breeding. However, they share features with anthropoids such as cohesive societies, female philopatry and individual recognition. Comparing social prosimians with anthropoids is crucial for understanding the evolution of reconciliation dynamics. Here we present the first study on reconciliation in a wild prosimian. We focused on the *Propithecus verreauxi* (sifaka) of the Berenty forest (southern Madagascar). We examined postconflict behaviour in the light of theoretical expectations based on potential costs and benefits of the individuals involved. Our results indicate that *P. verreauxi* can evaluate possible risks and benefits of engaging in postconflict reunions. Victims were most likely to interact affinitively with the aggressor after low-intensity aggression. Moreover, only the conflicts occurring outside the feeding context were reconciled. Such results are consonant with the fact that, in *P. verreauxi*, social dominance is translated more into feeding priority than into a framework of despotic relationships. In agreement with the valuable relationship hypothesis, *P. verreauxi* were more likely to reconcile with valuable partners: reconciliation preferentially occurred between subordinates and top-ranking individuals, and between animals sharing good relationships (high levels of affinitive behaviours). Over the short term, reconciliation in *P. verreauxi* seems to have an important role in reducing the probability of further attacks by the aggressor.

Keywords: conflict resolution; good relationships; lemur; Madagascar; *Propithecus verreauxi*; valuable relationship hypothesis; Verreaux's sifaka

Sociality is the norm in many mammalian species. The benefits of group living include lower risk of predation, better ability to defend food resources, and profit from sharing information (Wilson 2000; Alcock 2001). However, sociality also implies an increase of intragroup competition over resources, possibly leading to an escalation of aggressive behaviour (Moynihan 1998). Since conflicts jeopardize dyadic relationships and, consequently, social group cohesion (Cords 1992; Cheney & Seyfarth 1997; Wittig & Boesch 2005), animals have developed mechanisms to repair the disruption of dyadic relationships (Aureli et al. 2002).

One of the most discussed mechanisms of conflict resolution is reconciliation, defined as a form of affinitive interaction between former opponents, which make friendly contacts shortly after a fight (de Waal & van Roosmalen 1979). Reconciliation functions in restoring the relationship between the opponents after a conflict (de Waal 1986; Cords 1992; Cords & Thurnheer 1993; Aureli & de Waal 2000; Demaria & Thierry 2001; Wittig & Boesch 2005; Aureli & Schaffner 2006), in resuming the benefits associated with valuable relationships (Kappeler & van Schaik 1992). Moreover, postconflict reunions reduce the probability of further conflicts (Aureli et al. 1989; Aureli & van Schaik 1991; Cords 1992; Watts 1995a, b; Koyama 2001; Kutsukake & Castles 2001) and limit stress in the victim (Castles & Whiten 1998; Das et al. 1998; Aureli & Smucny 2000; Arnold & Whiten 2001; Butovskaya et al. 2005).

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Reconciliation has been found in primates (Aureli & de Waal 2000; Koyama & Palagi 2006; Thierry et al. 2008) and other social mammals (e.g. domestic goats, *Capra hircus*: Schino 1998; spotted hyaenas, *Crocuta crocuta*: Wahaj et al. 2001; bottlenose dolphins, *Tursiops truncatus*: Weaver 2003; domestic dogs, *Canis familiaris*: Cools et al. 2008; wolves, *Canis lupus*: Cordoni & Palagi 2008).

Within primates, this kind of conflict resolution has been found in all major groups of anthropoids: New World monkeys (e.g. captive squirrel monkeys, *Saimiri sciureus*: Pereira et al. 2000; captive white-faced capuchins, *Cebus capucinus*: Leca et al. 2002), Old World monkeys (e.g. captive patas monkeys, *Erythrocebus patas*: York & Rowell 1988; wild chacma baboons, *Papio ursinus*: Cheney et al. 1995; wild macaques, *Macaca* spp.: Aureli 1992; Cooper et al. 2005; captive guereza, *Colobus guereza*: Björnsdotter et al. 2000) and apes (captive and wild chimpanzees, *Pan troglodytes*: de Waal & van Roosmalen 1979; Arnold & Whiten 2001; captive bonobos, *Pan paniscus*: Palagi et al. 2004; wild mountain and captive lowland gorillas, *Gorilla beringei* and *Gorilla gorilla*: Watts 1995a, b; Cordoni et al. 2006).

Prosimian primates have been neglected for a long time in this area of research and, to our knowledge, postconflict behaviour has been investigated only in captive groups of Malagasy lemurs: *Eulemur fulvus*, *Eulemur macaco* and *Lemur catta* (Kappeler 1993; Rolland & Roeder 2000; Roeder et al. 2002; Palagi et al. 2005). Yet, comparing social prosimians with the best-known anthropoids is crucial for a better understanding of the evolution of conflict resolution mechanisms. In fact, lemurs (which retain ancestral traits such as a small brain and communication highly based on smell) contrast with anthropoids in various behavioural features, including female dominance, lack of sexual dimorphism regardless of mating system, and strict seasonal breeding (Martin 1990; Wright 1999). However, group-living lemurs share basic features with anthropoids such as cohesive multimale/multifemale societies, female philopatry (Pereira & Kappeler 1997) and individual recognition (Palagi & Dapporto 2006, 2007). Individual recognition is a prerequisite for reconciliation (Aureli et al. 2002).

Propithecus verreauxi (a species of sifaka, indriid family) is a diurnal and arboreal lemur that lives in multimale—multifemale groups spanning four to eight individuals: females are dominant over males and males are the main dispersing sex (Richard 2003). The individuals of a group move cohesively as foraging units (Richard 1974) and have to deal with tight energetic constraints, related to the energetically expensive locomotion (vertical leaping; Warren & Crompton 1997) combined with a low-quality diet mostly based on leaves (Norscia et al. 2006).

We investigated the occurrence of reconciliation in the *P. verreauxi* of the Berenty Reserve, southern Madagascar. By testing different predictions, we examined postconflict behaviour in the light of theoretical expectations based on potential costs and benefits of the individuals involved.

Prediction 1

Repairing valuable social bonds can be useful to both opponents (Aureli et al. 2002). However, victims should be more motivated to initiate postconflict reunions when the aggressor has a dominant status (Aureli & de Waal 2000; Wahaj et al. 2001). Owing to the risk of approaching the aggressor after a conflict, victims should be more likely to initiate reconciliation in tolerant species because of a lower risk of renewed attacks (Sterck et al. 1997).

Dominance in *P. verreauxi* is translated more into feeding priority than into general despotic relationships (Richard 1974). Because of this, the level of tolerance in this species should allow the victim (normally subordinate) to approach the attacker without taking much risk (benefits > costs). Thus, we predicted that in *P. verreauxi* victims are more likely than aggressors to make conciliatory approaches (prediction 1a). However, when the conflict of interest is over food, the risk may be high owing to the importance of the disputed resource (especially if monopolizable; cf. Isbell 1991; Verbeek & de Waal 1997; Majolo 2004). Consequently, reconciliation should occur at higher rates outside the feeding context (prediction 1b). Moreover, when aggression is severe, the danger (e.g. of bodily harm) can increase further, thus reducing the probability of a conciliatory approach (Schino et al. 1998; Aureli et al. 2002; Silk 2007). Reconciliation should then be higher after aggression of low intensity because this type of aggression can lower the risk of postconflict reunions (prediction 1c).

Prediction 2

The valuable relationship hypothesis (VRH) regards social relationships as investments (Cords & Aureli 1993, 2000; Cords & Thurnheer 1993; Cords 1997; Aureli & Schaffner 2006) and refers to how social partners benefit from one another. According to the VRH, reconciliation should occur more often when the opponents are mutually valued social partners because disturbance of a more valuable relationship entails a larger loss of benefits for both opponents (van Hooff 2001; Aureli et al. 2002).

In female-philopatric societies, social relationships can be particularly valuable for females: resident females, by forming strong bonds (also related to kinship), represent the core of the social group and they are the portal for group stability in the future (Silk et al. 2003). Consequently, females are likely to pay a higher price than males when a group is disrupted. Since *P. verreauxi* is usually characterized by female philopatry and male dispersal, we predicted reconciliation to be highest among females (prediction 2a).

Moreover, if the VRH is functional for *P. verreauxi*, we predicted that individuals sharing close relationships (measured by high frequency of grooming and body contact interactions; Preuschoft et al. 2002) would reconcile more frequently than subjects sharing weak relationships (prediction 2b).

Hierarchical relationships can also influence interindividual bonding and relationship quality. Hence, dominance rank is likely to affect the consequences of aggressive conflict and the relative interest of the contestants in conflict resolution (Preuschoft & van Schaik 2000). Consequently, we predicted dominance rank would influence the distribution of reconciliation in *P. verreauxi*, which lives in cohesive groups showing a clear-cut hierarchy (Richard 1974; prediction 2c).

Prediction 3

Recipients of aggression can be rather stressed after a conflict as a consequence of uncertainty associated with the risk of renewed attacks (Aureli et al. 1989). One of the functions of postconflict reunions is thought to be repairing social relationships, possibly removing the negative consequences of conflicts, such as the renewal of hostility (Silk 1996, 2007). Consequently, we predicted that in *P. verreauxi* the probability of further attacks by the aggressor (renewed aggression) would be reduced by postconflict reunions.

METHODS

Study Site and Groups

We conducted this study in the gallery forest of Berenty, a 200 ha reserve on the Mandrare River in southern Madagascar (for a complete description of the study site see Jolly et al. 2006). In particular, this research was conducted in the northern part of the forest called Ankoba (24.99°S, 46.29°E), a 40 ha secondary forest 50–60 years old, with canopy at 10–15 m (except for a few emergent acacias to more than 20 m). Usually, the site is characterized by two main climatic periods: a dry season from October to March and a wet season from April to September (Jolly et al. 2006).

We observed two groups of sifaka in the Ankoba area: group A and group B, of 10 and six individuals, respectively (Table 1). Kin relationships among group members were unknown. The individuals were well habituated to the presence of humans. Individual identification was based on sex and on distinctive external features (scars, size, missing fur patches, fur colour, facial traits; Jolly 1972).

Data Collection

We collected behavioural data during 4 months (November 2006–February 2007), in the wet season. The observations took place daily from dawn to dusk; we collected a total of 640 h of observation via focal animal sampling (40 h/individual). We collected data on all agonistic interactions among individuals via an all-occurrence sampling method (Altmann 1974). Systematic data collection was preceded by a training period that lasted until the observations by the various observers matched in 95% of cases (Martin & Bateson 1986). For each agonistic encounter we recorded: (1) opponents; (2) context (i.e. circumstance in which the aggression took place; for instance ‘feeding’, ‘resting’, ‘travelling’); (3) type of conflict (unidirectional or bidirectional); (4) aggressive behavioural patterns (mainly chasing, biting and slapping); and (5) submissive/frightened patterns (flee, vocalization, etc.). Since aggression could only be recognized by physical contact, the agonistic patterns were distinguished according to two stages of increasing intensity: stage 1, aggressive contact; stage 2, aggressive contact followed by submissive/frightened items.

After the last aggressive pattern of any given agonistic event, we followed the victim (as the focal individual) for a 15 min postconflict period (PC). Matched control observations (MCs) took place during the next possible day at

Table 1. Composition of the two *P. verreauxi* groups (A and B) observed in Berenty

Individuals	Age class	Sex class	Group	Attracted pairs	Dispersed pairs	Neutral pairs	CCT (%)
N	Adult	Male	A	3	1	1	40.00
S	Subadult	Female	A	3	1	3	28.57
SCR	Adult	Male	A	3	3	3	0.00
TB	Adult	Male	A	3	1	4	25.00
U	Adult	Male	A	8	0	21	27.59
UA	Adult	Male	A	4	0	2	66.67
GR	Adult	Male	A	8	1	5	50.00
MT	Adult	Female	A	2	0	2	50.00
OT	Adult	Male	A	5	0	0	100.00
P	Adult	Female	A	3	0	0	100.00
Mean CCT$_A$							48.78±10.24
BO	Adult	Male	B	11	3	4	44.44
BRA	Subadult	Female	B	3	0	4	42.86
BRO	Adult	Male	B	6	2	2	40.00
CL	Subadult	Male	B	10	2	7	42.11
CA	Adult	Female	B	3	0	6	33.33
BA	Adult	Female	B	4	2	2	25.00
Mean CCT$_B$							37.96±3.04
Mean CCT$_{A+B}$							44.72±6.51

The number of attracted, dispersed and neutral pairs collected per individual, with relative CCT (corrected conciliatory tendency, Veenema et al. 1994) are also shown.

the same time as the original PC, on the same focal animal, in the absence of agonistic interactions during the 15 min before the beginning of the MC and when the opponents had the opportunity to interact (de Waal & Yoshihara 1983; Kappeler & van Schaik 1992). To fulfil the second condition, we estimated by preliminary observations (30 h) that two individuals had the opportunity to interact easily when they were within 10 m of one another.

We collected a total of 161 PC–MC pairs on two groups of *P. verreauxi* (90 for group A and 71 for group B). The minimum number of PC–MC pairs recorded per focal animal was five (Table 1). For both PCs and MCs we recorded: (1) start time; (2) type of first affinitive interaction (body contact, grooming, touching, play, sexual contact); (3) minute of first affinitive contact; (4) initiator of the affinitive contact; and (5) partner identity. We also extracted background information on the relationship quality among individuals using affinitive interactions (grooming and contact sitting) collected by focal sampling (Altmann 1974).

Data Analysis

Reconciliation analysis was carried out at the individual level. For each animal we determined the number of attracted, dispersed and neutral pairs over all PC–MC pairs. In attracted pairs, affinitive contacts occurred earlier in the PC than in the MC (or they did not occur at all in the MC), whereas in dispersed pairs the affinitive contacts occurred earlier in the MC than in the PC (or they did not occur at all in the PC). In neutral pairs, affinitive contacts occurred during the same minute in the PC and the MC, or no contact occurred in either the PC or the MC. To avoid coding the same incident twice, for each individual we used only PC–MC pairs in which that individual was the focal animal, and entered them under its name. To evaluate individual reconciliation, we used Veenema et al.'s (1994) measure of corrected conciliatory tendency (CCT), defined as 'attracted minus dispersed pairs divided by the total number of PC–MC pairs'. Individual CCTs were used to determine the group mean CCT.

To investigate the influence of relationship quality on reconciliation, for each individual we first calculated the mean value of affinitive interactions for dyads (grooming and contact sitting) in which that selected individual was involved. Second, for each individual we divided dyads involving it into two quality classes (weak and close) by the following procedure: dyads showing both grooming and contact-sitting frequencies higher than the mean value of the selected individual were assigned to the close class; alternatively, dyads showing both grooming and contact-sitting frequencies lower than the mean value of the selected individual were assigned to the weak class. Afterwards, we calculated the mean CCT value that each subject showed with its partners belonging to close and weak relationship quality classes.

We used the Wilcoxon signed-ranks test, corrected for ties (Siegel & Castellan 1988) to check for the presence of reconciliation (attracted versus dispersed pairs) and the influence of relationship quality (close and weak), aggression context (feeding and no feeding) and intensity (stage 1, aggressive contact; stage 2, aggressive contact followed by submissive/frightened items) on reconciliation dynamics. We used the same test to evaluate (1) possible differences between aggressors and victims in initiating postconflict reunions and (2) whether the presence of conciliatory contacts influences the probability of renewed attacks by the same aggressor on the victim. We applied the Friedman's two-way analysis of variance (Siegel & Castellan 1988) to test for differences between the first postconflict affinitive patterns used to reconcile. The analyses were two tailed ($\alpha = 0.05$) and exact values were considered according to Mundry & Fischer (1998). The difference in the CCT distribution according to the sex class combination (female–female, FF; male–male, MM; and female–male, FM) was evaluated at the dyadic level via randomization procedures (one-way ANOVA; 10 000 shuffles; Manly 1997) using the freeware Resampling Procedures 1.3 (D. C. Howell, University of Vermont, Steamboat Springs, CO, U.S.A.).

We used all dyadic decided agonistic interactions recorded during the observation period to carry out hierarchical rank order analysis with the aid of MatMan version 1.0 software by Noldus (Noldus Information Technology, Wageningen, Netherlands; de Vries 1993). We used the David's score to determine each individual's rank position. This index calculates dominance ranks for individuals based on the outcomes of agonistic encounters with other group members, while taking the relative strength of their opponents into account (David 1987). The advantage of this approach is that it does not produce an illogical rank order when there are repeated interactions between pairs of group members, because minor deviations in the outcomes of such interactions do not affect individual ranks disproportionately (Gammell et al. 2003). Finally, we performed the K_r test following the rowwise partial matrix correlation (MatMan 1.0) to highlight the relationship between CCT levels and (1) rank position of the individuals and (2) rank distance measured as the absolute value of the difference between the David's scores of two individuals. As a control matrix, we used a dummy matrix of missing CCT values (referring to individuals that were never involved in a conflict; Hemelrijk 1990a, b).

RESULTS

We found a significant difference between attracted and dispersed pairs for both group A (attracted pairs > dispersed pairs; exact Wilcoxon signed-ranks test: $T = 0$, ties $= 1$, $N = 10$, $P = 0.004$) and group B (attracted pairs > dispersed pairs; $T = 0$, ties $= 0$, $N = 6$, $P = 0.031$). The mean CCT for group A ± SE was $\mathrm{CCT_A} = 48.78 \pm 10.24\%$ and for group B $37.96 \pm 3.04\%$. The CCT values did not differ significantly between the two groups (exact Mann–Whitney U test: $U = 25$, $N_A = 10$, $N_B = 6$, $P = 0.612$). Overall, the difference between attracted and dispersed pairs was extremely significant (attracted pairs > dispersed pairs; exact Wilcoxon signed-ranks test: $T = 0$, ties $= 1$, $N = 16$, $P < 0.001$). The overall mean CCT of both groups ± SE

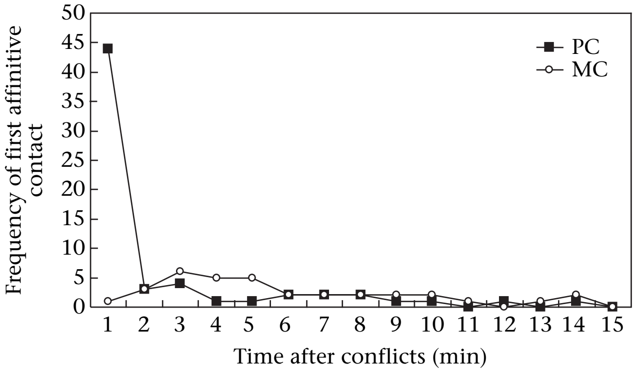

Figure 1. Temporal distributions of first affinitive contacts in postconflict (PC) and matched control (MC) periods for reconciliation.

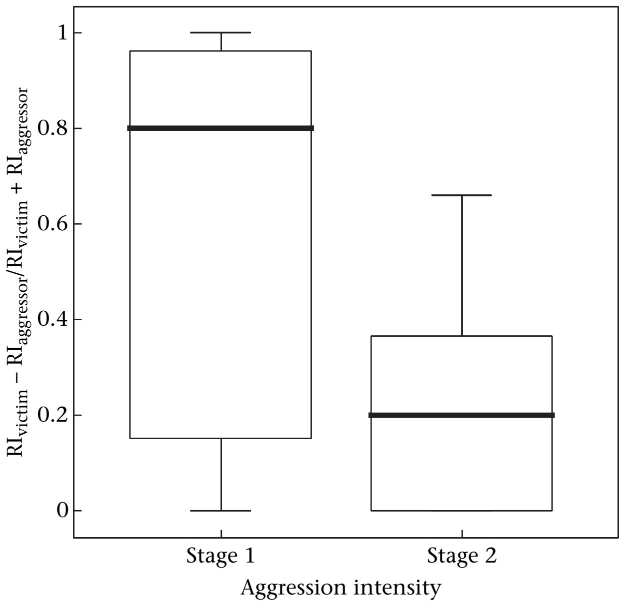

Figure 2. Frequency of reunion initiation (RI) by the victim (calculated as the ratio: $RI_{victim} - RI_{aggressor}/RI_{victim} + RI_{aggressor}$) according to aggression intensity (stage 1, low intensity; stage 2, high intensity). Solid horizontal lines indicate medians; box height corresponds to interquartile range; thin horizontal lines indicate range of observed values.

was 44.72 ± 6.51%. Figure 1 shows the temporal distributions of first affinitive contacts among PC−MC. The first postconflict affinitive patterns used to reconcile were body contact (48.5%), touching (31.5%) and grooming (20%). The use of such patterns in the reconciliation process did not differ significantly (exact Friedman test: $\chi^2_{r2} = 3.80$, $N = 16$, $P = 0.155$).

Prediction 1

The analysis of the initiator of postconflict reunions in attracted pairs showed a significant difference between aggressors and victims in group A (victim > aggressor; exact Wilcoxon signed-ranks test: $T = 2.5$, ties = 2, $N = 10$, $P = 0.039$) and a nonsignificant trend in group B (victim > aggressor; $T = 0$, ties = 1, $N = 6$, $P = 0.063$). Overall, the difference between aggressors and victims as reunion initiators (RI) was highly significant (victim > aggressor; $T = 4.5$, ties = 3, $N = 16$, $P = 0.002$). For the individuals involved in both stage 1 and stage 2 conflicts ($N = 9$), we calculated the following ratio $(RI_{victim} - RI_{aggressor})/(RI_{victim} + RI_{aggressor})$ to assess whether reunion initiation by the victim was influenced by the aggression intensity level. The victim initiated the postconflict reunion more frequently when the aggression intensity was low (stage 1; $T = 0$, ties = 2, $N = 9$, $P = 0.031$; Fig. 2).

The analyses considering the different aggression contexts (feeding and nonfeeding) and aggression intensity levels (stage 1 and stage 2) included only those subjects for which at least three PC−MC pairs were available in each of the four conditions ($N = 9$). For the different aggression contexts (feeding and nonfeeding), attracted and dispersed pairs did not differ significantly in the feeding context (exact Wilcoxon signed-ranks test: $T = 2.5$, ties = 4, $N = 9$, $P = 0.312$) but did differ in the nonfeeding context (attracted pairs > dispersed pairs; $T = 0$, ties = 2, $N = 9$, $P = 0.016$). The mean CCT for both groups ± SE was 24.07 ± 15.24% in the feeding context and 31.87 ± 6.74% in the nonfeeding context (Fig. 3a). For intensity level of agonistic encounters, the analysis revealed a significant difference between attracted and dispersed pairs for conflicts involving only aggressive contact (stage 1; attracted pairs > dispersed pairs; exact Wilcoxon signed-ranks test: $T = 0$, ties = 0, $N = 9$, $P = 0.004$) and a nonsignificant trend for conflicts involving aggressive contact followed by submissive/frightened items (stage 2; attracted pairs > dispersed pairs; $T = 1$, ties = 3, $N = 9$, $P = 0.063$). The overall mean CCT ± SE was 60.54 ± 10.78% for stage 1 and 14.37 ± 17.73% for stage 2 aggression. The CCTs recorded for stage 1 aggression were significantly higher than those recorded for stage 2 aggression ($T = 1$, ties = 0, $N = 9$, $P = 0.035$; Fig. 3b).

Prediction 2

For the sex class combinations (male−male, MM; female−male, FM; and female−female, FF) we detected no significant difference in the CCT distribution (one-way ANOVA via randomization: $F = 0.353$, $N_{FM} = 20$, $N_{FF} = 4$, $N_{MM} = 11$, $P = 0.704$). The animals for which it was possible to calculate the mean CCT for both weak and close relationships ($N_{TOT} = 14$; $N_A = 8$; $N_B = 6$) were included in the analysis to check the influence of relationship quality on reconciliation. Reconciliation occurred more frequently between individuals that shared a close relationship. Specifically, we obtained a statistical difference for group B (exact Wilcoxon signed-ranks test: $T = 0$, ties = 0, $N = 6$, $P = 0.031$; $CCT_{weak} \pm SE = 31.11 \pm 9.45\%$; $CCT_{close} \pm SE = 70.1 \pm 10.89\%$) and a nonsignificant trend for group A ($T = 3$, ties = 1, $N = 8$, $P = 0.078$; $CCT_{weak} \pm SE = 27.89 \pm 7.29\%$; $CCT_{close} \pm SE = 57.26 \pm 11.08\%$). When data were pooled, the difference was highly significant ($T = 4$, ties = 1, $N_{TOT} = 14$, $P = 0.002$; $CCT_{weak} \pm SE = 29.27 \pm 5.59\%$, $CCT_{close} \pm SE = 62.72 \pm 7.77\%$; Fig. 4).

We observed the presence of a linear hierarchy in group A ($h' = 0.600$, $P = 0.033$). The directional consistency

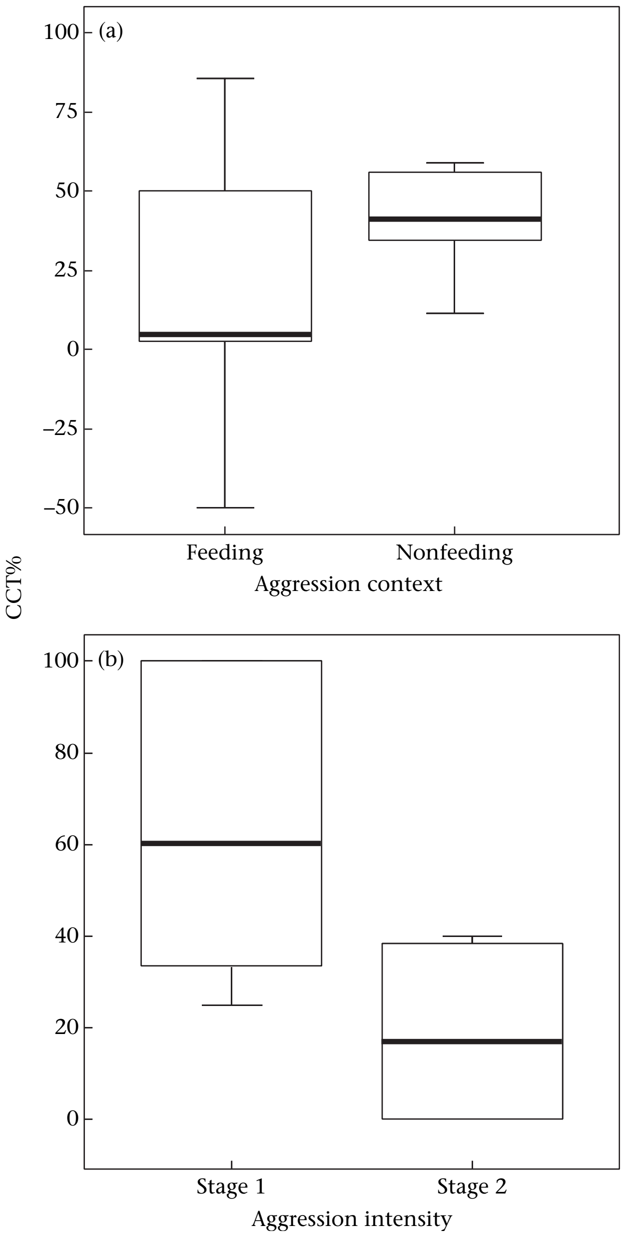

Figure 3. Corrected conciliatory tendency (CCT) levels according to (a) the aggression context (feeding versus nonfeeding) and (b) the aggression intensity (stage 1, low intensity; stage 2, high intensity). Solid horizontal lines indicate medians; box height corresponds to interquartile range; thin horizontal lines indicate range of observed values.

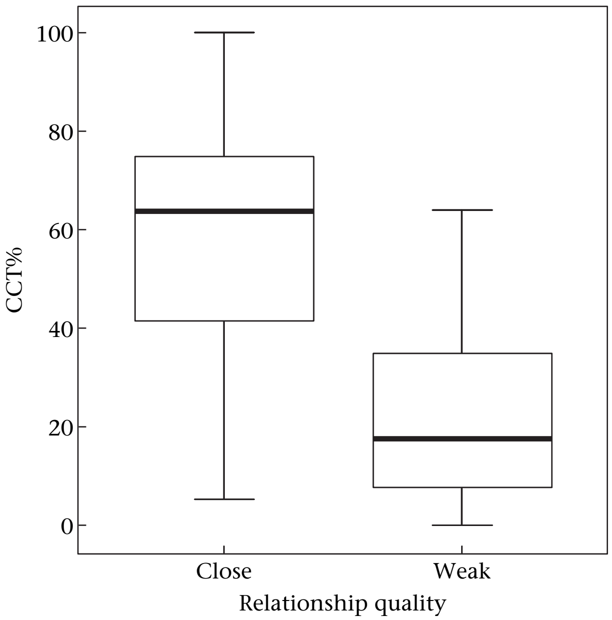

Figure 4. Corrected conciliatory tendency (CCT) levels according to relationship quality (close versus weak) between opponents. Solid horizontal lines indicate medians; box height corresponds to interquartile range; thin horizontal lines indicate range of observed values.

index (DC, the frequency in the relative direction of aggression) was 0.79 and the frequency of two-way relationships was 26.67%. For group B, we found a nonsignificant trend for the linear hierarchy ($h' = 0.89$, $P = 0.069$) with a DC value of 0.98 and the frequency of two-way relationships was 20.00%. The partial correlation with the K_r test did not reveal any relationship between the absolute rank position of the animals and the CCT levels (group A: $\tau_{K_r xy,z} = 0.035$, $N = 10$, $P = 0.040$; group B: $\tau_{K_r xy,z} = 0.117$, $N = 6$, $P = 0.268$). Instead, the same test revealed an extremely positive correlation (group A) and a positive correlation trend (group B) between rank distance (measured by the difference of David's score values) and CCT levels: the higher the rank distance, the higher the CCT level (group A: $\tau_{K_r\ xy,z} = 0.824$, $N = 10$, $P < 0.001$; group B: $\tau_{K_r\ xy,z} = 0.313$, $N = 6$, $P = 0.078$).

Prediction 3

The frequency of renewed attacks on the victim by the same aggressor was significantly lower in the presence than in the absence of reconciliation (exact Wilcoxon signed-ranks test: $T = 0$, ties $= 1$, $N = 7$, $P = 0.031$). Only the animals that underwent renewed attacks by the same aggressor within 15 min of the first act of aggression ($N = 7$) could be considered for the previous analysis (Fig. 5).

DISCUSSION

The study of conflict resolution has focused on anthropoid primates (only a few studies in the wild) and three species of captive prosimians (Kappeler 1993; Rolland & Roeder 2000; Schino 2000; Roeder et al. 2002; Palagi et al. 2005). Our study is the first to consider the occurrence of reconciliation in a group of wild prosimians, the arboreal lemur *P. verreauxi* (sifaka). Moreover, this is the first study on prosimian reconciliation that adopts an analysis at the individual level, thus allowing comparisons with other studies on primate and nonprimate species carried out by using the same approach.

Costs and benefits of disturbed relationships among group members are different between wild and captive

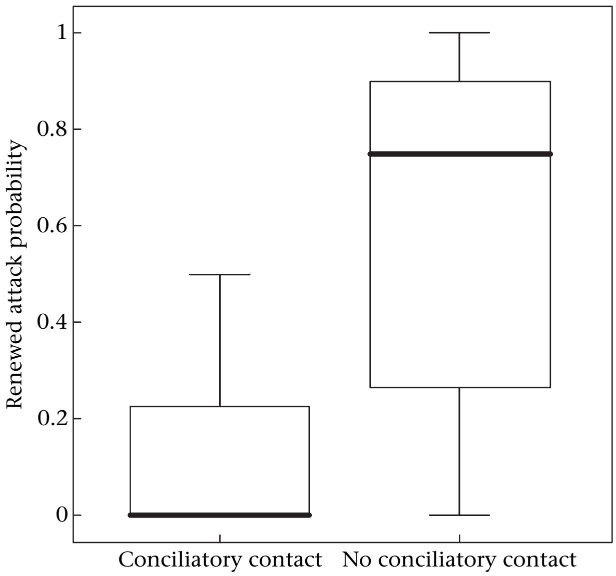

Figure 5. Probability of renewed attacks in the presence or absence of conciliatory contacts. Solid horizontal lines indicate medians; box height corresponds to interquartile range; thin horizontal lines indicate range of observed values.

set-ups. In the wild, interspecific competition, predation and seasonal bottlenecks in food availability contribute to shaping social bonding and counterdispersal strategies. For this reason, we have focused our discussion on results from wild studies, when available.

We found that reconciliation was present in both groups (A and B) of *P. verreauxi* under study with no preference for a specific conciliatory pattern (body contact, touching or grooming). Reconciliation has been found in *E. fulvus* but not in *E. macaco* and in three of the four groups of captive *L. catta* where postconflict reunions were investigated (Kappeler 1993; Palagi et al. 2005). The presence of reconciliation in *P. verreauxi* suggests that this species, characterized by relaxed interindividual relationships, is more similar to *E. fulvus* than to *L. catta* and *E. macaco* at least in terms of dominance style. The patchy occurrence of reconciliation in social prosimians suggests that the dominance style of the society, more than the phylogenetic closeness, can account for the presence of reconciliation. Consistently, across *Macaca* species (sharing similar social organization but different dominance style), a positive correlation has been found between the degree of group tolerance and the level of reconciliation (Thierry 1986, 2000; de Waal & Luttrell 1989). The fact that *P. verreauxi* can show a linear hierarchy does not imply that they have despotic relationships. Evidence that a structured hierarchy does not necessarily mean that tolerance levels are low comes also from several primate and nonprimate species (domestic goats: Schino 1998; hyaenas: Wahaj et al. 2001; wolves: Cordoni & Palagi 2008; macaques: Thierry 2000; chimpanzees: Wittig & Boesch 2003a; Palagi et al. 2006; bonobos: Palagi et al. 2004; Stevens et al. 2005).

In some species, the aggressor is responsible for initiating reconciliation (e.g. hyaenas: Wahaj et al. 2001; primates such as moor macaques, *Macaca maura*: Matsumura 1996; patas monkeys: York & Rowell 1988; rhesus monkeys, *Macaca mulatta*: de Waal & Ren 1988; sooty mangabeys, *Cercocebus atys*: Gust & Gordon 1993). In other species, the victim is more likely to initiate the postconflict reunion (e.g. domestic goats: Schino 1998; primates such as chimpanzees: de Waal 1989; stumptailed macaques, *Macaca arctoides*: de Waal & Ren 1988; black- and white quereza monkeys: Björnsdotter et al. 2000). It has been hypothesized that such variation results from differences in dominance styles (de Waal & Luttrell 1989; Matsumura 1996). Victims should be more inclined to achieve reconciliation than aggressors, especially in tolerant species, where the risk of renewed attacks is limited (Sterck et al. 1997). The fact that in *P. verreauxi*, victims (usually subordinate individuals) were the most responsible for postconflict affinitive interactions (prediction 1a confirmed) provides further evidence of the tolerant nature of relationships in this species. This finding matches with the result found in *E. fulvus* (Kappeler 1993) but contrasts with the situation described by Palagi et al. (2005) for *L. catta*, a well-known despotic species (Jolly 1966; Pereira & Kappeler 1997). In fact, in the only group of *L. catta* where reconciliation was found, aggressors were the most involved in conciliatory approaches, possibly because of the victims' fear of renewed aggression (Palagi et al. 2005). As in captive *E. fulvus*, in *P. verreauxi* conciliatory approaches were particularly frequent after low-intensity conflicts (prediction 1c confirmed), thus suggesting that the individuals evaluate the possible risks before engaging in postconflict reunions. Risk evaluation is also suggested by the absence of reconciliation in the feeding context (prediction 1b confirmed). In fact, conflicts over food can be particularly dangerous since they can fall into the category of 'contest food competition' (van Schaik & van Noordwijk 1988; Ricklefs 2001). Conflicts over food were rarely followed by postconflict reunions in wild macaques (Aureli 1992; Matsumura 1996; Majolo 2004). In wild chimpanzees, postconflict reunions after conflicts over food were preferentially initiated by losers, possibly because the conciliatory approach increases the probability of the loser obtaining food from the aggressor (Wittig & Boesch 2003b): in this case, the benefit of food sharing prevails over the cost of renewed aggression. In general, the benefit of reconciliation is not always able to compensate for the high risk related to postconflict approaches because unresolved conflicts over food do not necessarily cause long-term detrimental effects on the social relationship between former opponents (Aureli 1992). The consequences of food-related aggression may be limited to the displacement from the food source without endangering the relationship between the opponents (Verbeek & de Waal 1997), especially in those species where dominance is expressed in terms of feeding priority such as *P. verreauxi*.

Benefit evaluation is an important part of conflict resolution, which may be more or less effective depending on the individuals involved (Aureli & de Waal 2000). The VRH, which states that individuals are particularly likely to reconcile with partners that are valuable or attractive to them in other social contexts (Cords & Aureli 1993), has found support in the wild (e.g. chimpanzees: Watts 2006; gorillas: Watts 1995a; white-faced capuchins: Manson et al. 2005; macaques: Aureli et al. 1997).

In female-philopatric societies, females have more interest in establishing long-term relationships with other females (Silk et al. 2003) and should be more involved in postconflict reunions than males. Accordingly, in wild macaques, females showed a higher conciliatory tendency than males (bonnet macaques, *Macaca radiata*: Cooper et al. 2007; Assamese macaques, *Macaca assamensis*: Cooper et al. 2005). In *P. verreauxi* we found no evidence of higher reconciliation levels among females, compared to the other sex class combinations (prediction 2a not confirmed). This result could be linked to the fact that the data collection period included the mating season, a period in which the social dynamics of *P. verreauxi* can vary (Brockman & Whitten 1996; Brockman 1999). In seasonally breeding species such as lemurs, the mating season is a period in which competition for mating partners increases, affecting social relationships and possibly reducing female social bonding (D'Amato et al. 1982; Mehlman & Chapais 1988). In the mating season, affinitive behaviours between males and females increased in the *P. verreauxi* of Berenty (E. Palagi & I. Norsica, unpublished data), possibly influencing the baseline distribution of conciliatory contacts across sex combinations. In wild Japanese macaques, *Macaca fuscata*, for example, the conciliatory tendency among females was significantly reduced in the mating season, thus supporting the above scenario (Majolo & Koyama 2006).

One of the main aspects considered by the VRH is the quality of relationships among group members. Indeed, individuals that spend much time in affinitive behavioural exchange are likely to be valuable partners, because they tend to tolerate, support and protect one another in different social contexts (Cords 1997). In this view, friendship (measured as the baseline level of affinitive interactions, body contact and grooming), by positively influencing the value of a relationship, can increase the reconciliation rates (van Hooff 2001). This scenario has been supported by some studies on wild primates (Aureli et al. 1997; Arnold & Whiten 2001; Wittig & Boesch 2003c; Watts 2006). Our results on *P. verreauxi* further support the above hypothesis because the individuals sharing strong bonds (close relationships) did reconcile most frequently.

Beyond sex and friendship, dominance rank in the hierarchy can potentially influence the value of relationships among group members. Indeed, power asymmetry determines who may interact with whom (Preuschoft & van Schaik 2000). In *P. verreauxi*, we found that rank distance between group members (and not the absolute dominance ranking position) affected the CCT distribution: the higher the rank distance, the higher was the reconciliation level between individuals.

Silk et al. (1996) and Judge (1991) have reported the opposite phenomenon in wild baboons, *Papio cynocephalus ursinus*, and captive pigtailed macaques, *Macaca namestrina*, respectively. Such species show matrilinear hierarchies and related females occupy adjacent ranking positions. In both cases, individuals close in rank reconciled more frequently than those ranked distantly to one another. However, this tendency was driven by kin-based preference in baboons (Silk et al. 1996) and it was independent of kinship in pigtailed macaques (Judge 1991). To date, no evidence has been provided on how dominance status is acquired in *P. verreauxi* and if dominance is somehow related to kinship. However, the small size of the groups (foraging units of 4–8 individuals; Richard 2003; Lewis 2005) does not allow the formation of clans of related females or large subunits of close-ranking individuals.

Reconciling with top-ranking individuals can be valuable for subordinates when dominance steepness and despotism are low (cf. Kappeler 1993; Thierry 2000). Consequently, the finding that low-ranking *P. verreauxi* reconciled preferentially with top-ranking group members indicates that the dominance gradient is not too steep. Otherwise, subordinates would be inhibited from approaching or making contact with dominant group members because of the costs of possible subsequent attacks. The possibility of subordinates reconciling with high-ranking individuals has been reported in captive and wild macaques, in which the hierarchy is stable but the dominance gradient is not severe (Thierry 1990; Petit et al. 1992; Matsumura 1996).

The tolerance in a society reduces but does not eliminate the possibility of renewed attacks by the former aggressor (Aureli & de Waal 2000). One of the functions of reconciliation (already suggested for different anthropoid species) is reducing the negative consequences of hostility renewal (Silk et al. 1996; Silk 2007). In *P. verreauxi* the frequency of renewed attacks on the victim by the same aggressor was significantly lower in the presence than in the absence of reconciliation. Moreover, in *L. catta* the probability of renewed attacks increased in the postconflict period, in the absence of reconciliation (Kappeler 1993). These results converge in indicating that postconflict reunions are effective in limiting aggression spreading in prosimians, as they can be in anthropoids (van Hooff 2001; Aureli & Schaffner 2006).

In conclusion, by clarifying reconciliation patterns in wild *P. verreauxi*, we have shed light on the mechanisms of conflict resolution that may be used by group-living prosimians as a function of social tolerance.

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etologia

La strategia della pace

Ivan Norscia ed Elisabetta Palagi

La riconciliazione è un comportamento ben noto in molti mammiferi sociali, e in particolare nei primati. Molti studi hanno confermato questa modalità di interazione tra i macachi, tra i babbuini, e nelle tre specie antropomorfe, scimpanzé, gorilla, bonobo. Poco si sa invece delle capacità riparatorie delle proscimmie, animali difficili da osservare per le loro abitudini notturne e solitarie. Per colmare questa lacuna, ricercatori italiani hanno osservato per quattro mesi il comportamento del lemure bianco in Madagascar, una specie sociale in cui le femmine sono il sesso forte. Scoprendo che nelle società di queste proscimmie fare la pace è una pratica molto diffusa.

Chi più, chi meno, gli animali che vivono in gruppo conoscono diverse strategie per gestire i conflitti tra gli individui. Si può intervenire a monte per evitare di arrivare ai ferri corti, ma si può anche tornare "amici come prima" a litigio concluso. Quando la prima tattica fallisce e gli avversari scelgono di fronteggiarsi in uno scontro aperto, la successiva rappacificazione può riportare l'equilibrio nella comunità evitando che il conflitto dilaghi al suo interno. È un comportamento ben noto a molte specie animali: sanno fare la pace le capre domestiche (*Capra hircus*), le iene (*Crocuta crocuta*), i delfini (*Tursiops truncatus*), il cane domestico (*Canis familiaris*), il lupo (*Canis lupus*) [1]. Tuttavia, è tra i primati, mammiferi sociali per antonomasia, che la capacità di riappacificarsi è stata maggiormente studiata, anche se con qualche lacuna. Sappiamo molto sui comportamenti riparatori dei macachi, dei babbuini e delle tre antropomorfe sociali (scimpanzé, gorilla e bonobo) mentre abbiamo poche informazioni sulla riconciliazione nelle proscimmie. La ragione principale è che la maggior parte delle proscimmie (che vivono in Asia e Africa) conduce vita notturna e solitaria. Tuttavia, alcune specie di lemuri (le proscimmie del Madagascar) vivono in gruppi coesi, prerequisito essenziale per studiare un fenomeno tipicamente sociale come la riconciliazione.

Gli unici studi sull'argomento sono stati condotti su tre specie di lemuri in cattività (*Lemur catta*, *Eulemur fulvus*, *Eulemur macaco*) e hanno prodotto risultati controversi e non risolutivi: la riconciliazione risulta presente solo in alcune delle colonie studiate oppure non è presente affatto.

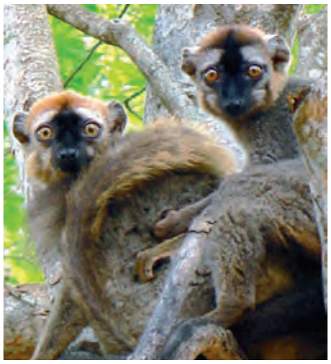
Per colmare questa lacuna, abbiamo effettuato uno studio, in natura su una proscimmia sociale del Madagascar: il lemure bianco, appartenente alla specie *Propithecus verreauxi* (comunemente chiamato sifaka e caratterizzato da una tipica locomozione a salti, con postura verticale (*vertical climbing and leaping*).

Vivere in società

Alcuni mammiferi sono solitari, altri vivono in gruppi stabili e tutti, nessuno escluso, si trovano a dover socializzare in almeno una fase della vita. Questa fase è l'allattamento, esclusivo dei mammiferi, durante il quale i piccoli si trovano a condividere uno spazio in comune con i fratelli e a competere per l'accesso al latte materno. Anche quando la femmina partorisce un solo piccolo per volta, un nucleo sociale primario esiste sempre: è quello composto da madre e figlio, che interagiscono fino a che lo svezzamento non è completato. È lì che ha origine la capacità di "fare gruppo" [2].

Una società è facilmente riconoscibile perché formata da individui costantemente in rapporto tra loro. Ve ne sono di vari tipi che si distinguono per tre aspetti fondamentali, che spesso vengono confusi anche dagli "addetti ai lavori": la struttura, l'organizzazione e il sistema di accoppiamento. La struttura sociale identifica il livello di coesione del gruppo: ci sono società cosiddette a "fissione-fusione" in cui sottogruppi diversi si separano e riuniscono a seconda delle esigenze. Maschi e femmine possono riunirsi nella stagione degli amori e più gruppi che si procacciano il cibo separatamente durante il giorno possono radunarsi in enormi mandrie per dormire più sicuri durante la notte. L'organizzazione sociale identifica il rapporto tra maschi e femmine e le dimensioni del gruppo: si può avere un harem, con un solo maschio adulto e più femmine riproduttive, una società con diversi maschi e femmine adulti (*multimale-multifemale*), oppure un gruppo familiare, con un

Sifaka (Propithecus verreauxi*) nella foresta galleria di Berenty, in fase di moving durante la ricerca mattutina di cibo. Sotto, una madre gidro (nome comune di questo lemure) trasporta sul dorso un piccolo di due mesi circa; al centro una madre maki* (Lemur catta*) con il suo piccolo durante il* feeding *(alimentazione) su fiori e frutti maturi. In basso* Lepilemur mustelinus*, un lemure della famiglia dei Lepilemuridi tipicamente notturno e solitario. Durante il giorno riposa nelle cavità dei tronchi.*



maschio e una femmina adulti e la loro prole. Il sistema di accoppiamento si riferisce, invece, al livello di "fedeltà" dei maschi e delle femmine: esistono animali che vivono in coppie monogame, cioè in cui una femmina si accoppia con lo stesso maschio per più stagioni riproduttive (come accade in alcuni lemuri), e società "promiscue" in cui maschi (poliginia), femmine (poliandria) o entrambi (poligamia) scelgono più partner sessuali per massimizzare il loro successo riproduttivo. La poligamia è, in effetti, la situazione più frequente in natura [3]. Esistono, infine, società dispotiche in cui il rispetto della gerarchia viene imposto con la forza da parte di un individuo detto "alfa", generalmente maschio (è il caso del lupo o del gorilla) e società più egalitarie, con un più alto grado di tolleranza e in cui i rapporti tra gli individui, più o meno definiti in una gerarchia stabile, sono rilassati (è il caso del bonobo).

La gestione dei conflitti

Nel corso della loro evoluzione, iniziata più di 100 milioni di anni fa, molte specie di mammiferi hanno sviluppato la capacità

*Due sifaka su un kilimbasaha (*Pithecellobium dulce*, principale specie vegetale della foresta secondaria di Ankoba, nella parte nord della riserva di Berenty) impegnati in una sessione di* grooming *(spulciamento) reciproco, durante la pausa pomeridiana che segue il feeding mattutino.*

di vivere in "comunità" coese. Tra questi, i canguri (*Macropus* spp) (che fanno parte dell'antico gruppo dei marsupiali), le balene (*Hyperoodon ampullatus*), i lupi (*Canis lupus*), le iene (*Crocuta crocuta*), gli elefanti (*Loxodonta africana*) e la maggior parte dei primati [4].

La vita sociale presenta indubbiamente dei vantaggi, che derivano principalmente dalla cooperazione tra i membri di uno stesso gruppo: la maggiore protezione dai predatori, la possibilità di sparpagliarsi per cercare il cibo in un territorio più ampio e il beneficio di condividere informazioni ed esperienza. Ma la vita in gruppo ha anche un costo, dovuto alla maggiore competizione tra gli individui, che possono entrare in conflitto per uno spazio ambito (magari all'ombra di un'acacia spinosa nella soleggiata savana), un cibo particolarmente gradito (come i frutti zuccherini dell'unico tamarindo disponibile nel territorio), o per l'accesso alle femmine (per il quale i maschi possono battersi ferocemente) [4]. Gli animali non umani però hanno evoluto diversi meccanismi per gestire l'aggressività, che possono agire a priori, in modo da evitare che un conflitto d'interesse si trasformi in un conflitto reale, oppure a posteriori, "riparando" relazioni sociali interrotte da un episodio aggressivo.

In parziale disaccordo con il modello individuale lorenziano, che interpretava l'aggressività come un aspetto quasi "insalubre" della convivenza (derivante da isolamento sociale e/o da una combinazione di fattori genetici e fisiologici), gli etologi contemporanei considerano gli scontri come una parte integrante della vita sociale. Secondo il modello relazionale proposto da Frans de Waal, gli individui che vivono all'interno di un gruppo sociale hanno tre possibilità: evitarsi, tollerarsi o entrare in conflitto [5]. Alcuni comportamenti affiliativi, cioè amichevoli, come il gioco, vengono utilizzati per aumentare la tolleranza all'interno del gruppo e ridurre i livelli di tensione, specialmente durante i pasti o gli accoppiamenti, cioè quando c'è in ballo una risorsa importante e contesa. È tuttavia inevitabile che, prima o poi, si verifichi un scontro aggressivo tra qualche membro del gruppo: è qui che scatta la necessità, per i due opponenti, di riconciliare e ripristinare la relazione sociale bruscamente interrotta. La riconciliazione ha anche la funzione di evitare che il conflitto dilaghi all'interno del gruppo. Un fenomeno abbastanza comune, infatti, è quello della "redirezione", in cui un individuo che ha subito un'aggressione si "rivale" su un terzo individuo, aggredendolo per sfogare stress e frustrazione. Quando i livelli di stress e di scontri aggressivi diventano troppo alti, si può giungere alla disgregazione o, addirittura, all'annientamento del gruppo sociale.

Un epilogo di questo tipo è stato documentato da Jane Goodall negli anni Ottanta in un gruppo di scimpanzé (*Pan troglodytes*) di Gombe, in Tanzania. I membri della comunità sociale, non più in grado di gestire i conflitti generati dalla scarsità di cibo, si divisero in due fazioni e iniziarono a fronteggiarsi in vere e proprie guerre, con agguati e coalizioni per uccidere gli individui della parte avversaria (*coalitionary killing*) [6]. Alla fine, della grande comunità originaria non rimase che uno sparuto gruppo di individui. È evidente, tuttavia, che l'eventualità che un conflitto per la difesa dei singoli interessi culmini nella polverizzazione del gruppo è rara, proprio perché la selezione naturale ha favorito i meccanismi di prevenzione e recupero di situazioni che generano elevata instabilità sociale.

La ricerca

Il *Propithecus verreauxi* (sifaka) è un lemure diffuso soprattutto nel Sud del Madagascar. Vive in gruppi coesi di 6-8 individui che si spostano compatti, delle vere e proprie "unità di foraggiamento" [7,8]. Come nella maggior parte dei lemuri sociali, anche tra i sifaka le femmine sono il sesso "forte". La loro dominanza però si manifesta nella priorità di cui godono nell'accesso al cibo, piuttosto che attraverso comportamenti aggressivi. Questo è un chiaro segnale che la società dei sifaka è caratterizzata da alti livelli di tolleranza.

*Alberi di Tamarindo (*Tamarindus indica*) che segnano l'inizio della foresta galleria di Berenty, sulle rive del fiume Mandrare che attraversa la regione semidesertica Antandroy, nel Sud del Madagascar. Sotto, sifaka su una Fatsiolotra (*Allouadia procera*), pianta endemica che domina la foresta spinosa.*

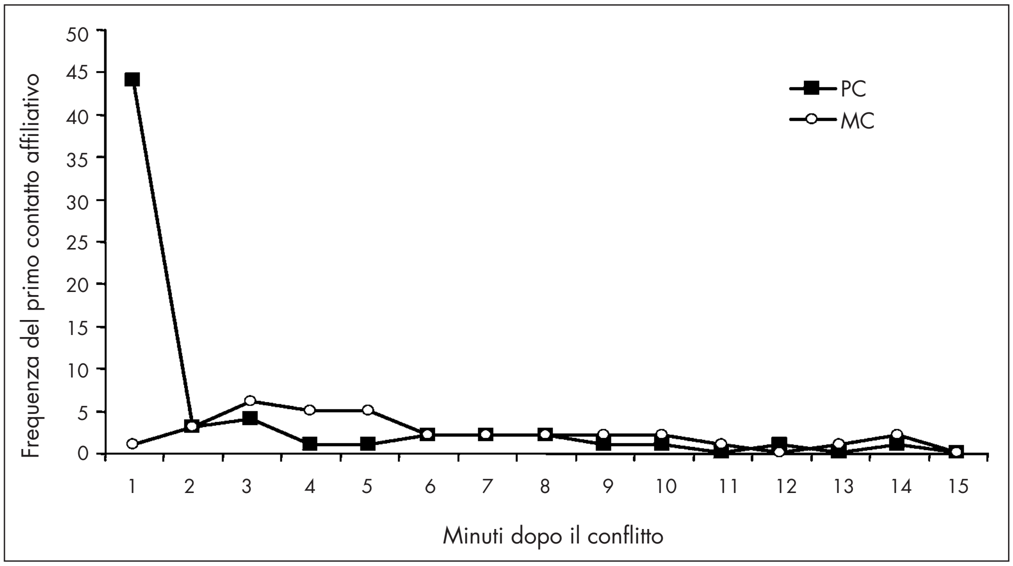


Fig. 1. Contatti affiliativi (frequenza) tra i due contendenti in presenza (PC) e in assenza (MC) di conflitto. Il contatto riconciliatorio avviene prevalentemente entro il primo minuto dallo scontro.

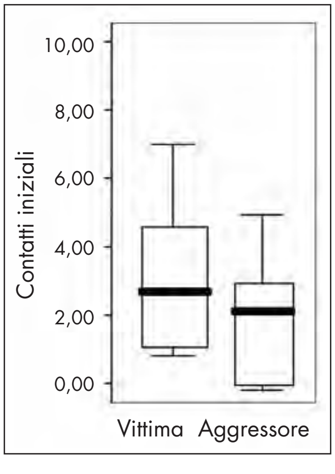
Lo scopo della nostra ricerca era duplice: capire se la capacità di fare pace ha radici antiche all'interno dei primati (basi biologiche della riconciliazione) e verificare se tale capacità è più legata alla vicinanza filogenetica delle specie o alle caratteristiche del gruppo sociale, come il livello di dispotismo e il tipo di gerarchia.

Come prima cosa, abbiamo individuato i gruppi sociali che avremmo osservato in base a un rigido protocollo. Nel nostro caso abbiamo seguito nella foresta galleria di Berenty (Madagascar meridionale) due gruppi di sifaka, denominati A e B e composti rispettivamente da 10 e 6 animali [9]. La raccolta dati è stata possibile anche grazie a Daniela Antonacci, studentessa di dottorato che, dopo una prima fase di training, è rimasta quattro mesi sul campo per completare le osservazioni. Ciascun individuo è stato identificato in base a differenze di sesso, taglia, colorazione del pelo e alla presenza di tagli o cicatrici.

Alla fine del periodo di studio, durato quattro mesi, abbiamo accumulato 640 ore di osservazione (40 ore per individuo). Tutti i comportamenti che non è stato possibile attribuire con certezza a un individuo (per ragioni di visibilità nella fitta vegetazione della foresta) sono stati esclusi dalle analisi. I comportamenti da rilevare sono codificati mediante un etogramma (una lista di azioni definite sulla base studi precedenti e osservazioni preliminari) e suddivisi in stati ed eventi. Gli stati sono azioni che hanno una durata apprezzabile (o, in termini pratici, cronometrabile) di almeno 20 secondi, come mangiare (*feeding*), spulciarsi (*grooming*), riposare (*resting*), stare seduti in contatto (*contact sitting*) o ingaggiare una lotta, vera o per gioco (*aggressive/play wrestle*). Gli eventi sono invece azioni istantanee, come uno schiaffo aggressivo (*aggressive slap*) o uno dato per gioco (*play slap*), un salto da un ramo all'altro (*leaping*) e così via.

Il metodo utilizzato per verificare la presenza di riconciliazione è chiamato PC-MC e prevede di osservare ogni individuo per un tempo stabilito a priori (nel nostro caso 15 minuti) in due condizioni diverse, cioè sia in presenza sia in assenza di conflitto. In pratica, dopo uno scontro tra due individui si osserva la vittima nel periodo post-conflitto (PC, *post-conflict*) e si registrano tutti i comportamenti previsti dall'etogramma e le interazioni con gli altri membri del gruppo. Lo stesso tipo di osservazione deve essere ripetuto (osservazione di controllo, MC, *match control*) sulla vittima in uno dei giorni successivi, alla stessa ora e nelle stesse condizioni ambientali (sole o pioggia, ad esempio) in assenza di conflitti. La doppia analisi permette di valutare se i rapporti tra gli individui coinvolti nella lite cambiano dopo lo scontro. Se, dopo un conflitto, i contatti affiliativi (amichevoli) tra aggressore e vittima (cioè il *grooming* e il *contact sitting*) aumentano, la coppia PC-MC si dice "attratta"; se i rapporti positivi diminuiscono, si dice "dispersa" mentre se non c'è nessun cambiamento apprezzabile si dice "neutra". Si stabilisce che è presente la riconciliazione se le coppie attratte sono significativamente più numerose (in termini statistici) delle coppie disperse.

Fig. 2. Box plot relativo all'inizio del contatto conciliatorio. La vittima è quella che più frequentemente cerca il contatto per ripristinare la relazione interrotta dallo scontro (test per dati appaiati di Wilcoxon; T = 4.5, ties =3, N = 16, P = 0.002). Le linee orizzontali nere indicano le mediane; l'altezza dei box indica l'intervallo interquartile e le linee verticali indicano l'intervallo dei valori riportati sulle ordinate.



Risultati

Possiamo affermare che tra i sifaka la riconciliazione è una prassi diffusa. Alla fine del periodo di osservazioni abbiamo raccolto 161 coppie PC-MC, 90 per il gruppo A e 71 per il gruppo B. Dopo un conflitto, gli animali hanno dunque molte interazioni positive (numero di coppie attratte significativamente maggiore di quelle disperse; Test per dati appaiati di Wilcoxon; T=0, ties=1, N= 16, P=0.001). Inoltre, i contatti riconciliatori avvengono prevalentemente entro il primo minuto dall'aggressione, mentre i normali contatti affiliativi (indipendenti dal conflitto) sono "spalmati" su tutto il periodo di osservazione (Fig. 1).

Una volta appurato che la riconciliazione è presente nei gruppi sifaka, resta da scoprire se tutti gli individui la praticano allo stesso modo. Per poter dare una risposta occorre innanzitutto misurare la "propensione" a riconciliare. A tal fine si usa un indice percentuale, chiamato CCT (Corrected Conciliatory Tendency), espresso come la differenza tra coppie attratte e disperse divisa per la somma totale delle coppie (attratte, disperse e neutre). Così si può assegnare un "voto" alla capacità di far pace. Ci sono primati che riconciliano a frequenze molto basse (per esempio i gorilla, con una CCT poco al di sopra del 10 per cento) e altri a frequenze più alte (come gli scimpanzè e i bonobo, in cui le CCT possono raggiungere il 50 per cento a seconda delle classi di età e di sesso). I sifaka mostrano livelli conciliatori piuttosto alti, con CCT che superano il 40 per cento.

Confrontando le CCT individuali abbiamo riscontrato che le vittime iniziano il processo di riconciliazione più frequentemente rispetto a quanto fanno gli aggressori (Fig. 2), probabilmente perché di solito si tratta di individui subordinati, ai quali conviene mantenere buoni rapporti con i dominanti. A sostegno di ciò, abbiamo riscontrato che gli individui di più basso rango riconciliano maggiormente con chi si trova all'apice della gerarchia. Questo comportamento è in accordo con l'ipotesi delle relazioni "proficue" (*valuable relationship hypothesis*), secondo cui gli individui hanno maggiore interesse a ripristinare i rapporti che possono risultare

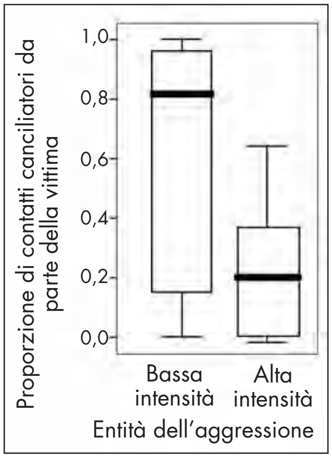
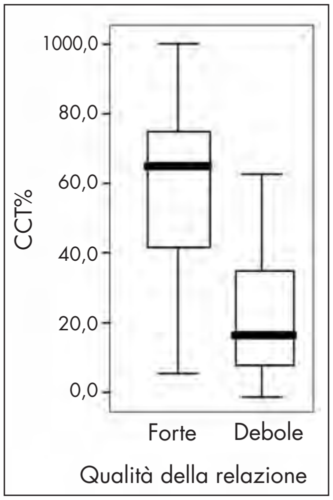


Fig. 3. Box plot relativo alla proporzione di contatti conciliatori iniziati dalla vittima, misurati come il rapporto tra differenza (al numeratore) e somma (al denominatore) tra i contatti iniziati dalla vittima e quelli iniziati dall'aggressore (asse delle ordinate). Gli scontri di più lieve entità vengono riconciliati in misura significativamente maggiore (test per dati appaiati di Wilcoxon; T=0, ties=2, N= 9, P=0.031). Le linee orizzontali nere indicano le mediane; l'altezza dei box indica l'intervallo interquartile e le linee verticali indicano l'intervallo dei valori riportati sulle ordinate.

Due sifaka giocano impegnati in una sessione di play wrestle *(lotta per gioco) e* acrobatic play *(gioco acrobatico) durante un momento di relax.*

Primo piano di un sifaka. Il colore del cappuccio marrone può variare dal grigio chiaro al rossastro-marrone scuro e questo permette una prima identificazione dell'animale all'interno del gruppo.

Fig. 4. Box plot relativo alla tendenza conciliatoria (CCT) registrata tra individui con legami più forti (buone relazioni) e quelli con legami più deboli. I sifaka che hanno maggiori contatti "amichevoli" in condizioni normali riconciliano in modo significativamente maggiore rispetto a quelli che hanno scarse relazioni positive (test per dati appaiati di Wilcoxon; T=4, ties=1, N= 14, P=0.002). Le linee orizzontali nere indicano le mediane; l'altezza dei box indica l'intervallo interquartile e le linee verticali indicano l'intervallo dei valori riportati sulle ordinate.



più vantaggiosi nel futuro (alleanze vincenti, protezione o accesso al cibo) [10]. Uno dei casi più eclatanti è quello del gorilla di pianura. In questo caso la riconciliazione è presente solo tra le femmine e il *silverback* (maschio alfa del gruppo): in una struttura ad harem dove il maschio dominante è in grado di imporre la propria volontà con la forza, le femmine puntano soprattutto a tenersi buono il *silverback*.

Riconciliare comporta indubbiamente dei rischi soprattutto per la vittima che, riavvicinandosi all'aggressore per un contatto affiliativo, rischia di subire nuove aggressioni. Ma i sifaka sembrano in grado di valutare costi e benefici, privilegiando gli individui "di maggior valore" (dominanti) e adottando qualche cautela: la vittima cerca il contatto conciliatorio più frequentemente dopo un'aggressione di lieve entità (nel nostro caso non seguita da vocalizzazioni di paura) (Fig. 3). Inoltre, quei sifaka che si scambiano, in condizioni di pace, più contatti affiliativi sono anche quelli che, in caso di scontro, riconciliano con più facilità. Questi risultati sono in accordo con l'ipotesi delle buone relazioni (*good relationship hypothesis*) secondo la quale gli individui che hanno migliori rapporti hanno maggior propensione a far pace [5]. In poche parole, gli "amici" fanno pace più facilmente degli estranei anche tra le proscimmie.

La riconciliazione, oltre a preservare la stabilità del gruppo, può offrire vantaggi immediati per la vittima, tra cui una minore probabilità che il dominante reiteri l'aggressione. Nei sifaka infatti la probabilità di un nuovo attacco si riduce quando interviene un contatto conciliatorio.

Considerazioni finali

La capacità di far pace è probabilmente nata con i primati, perché si ritrova anche in quelli più antichi, come le proscimmie (comparse tra 60 e 80 milioni di anni fa), che già sanno valutare costi e benefici dalla riconciliazione. I lemuri sono specie ideali per valutare il legame tra tipo di società e riconciliazione, perché privi di sovrastrutture culturali. Nei primati "superiori", invece, possono subentrare elementi di confondimento

Un momento delle osservazioni, mentre gli animali sono in fase di riposo. Da sinistra a destra: Ivan Norscia, Daniela Antonacci e uno studente universitario (foto di Elisabetta Palagi).

dovuti alla complessità psicologica dei comportamenti. In generale, la riconciliazione è più diffusa nelle società poco gerarchizzate, mentre lo è molto meno nelle società dispotiche (un trend non sempre rispettato nei primati più evoluti, proprio per la complessità psicologica delle loro azioni). I sifaka riconciliano con gli individui di più alto "valore" (i dominanti o gli "amici") e prevalentemente quando è il rischio è basso (cioè l'aggressione è stata lieve), secondo una tipica valutazione di "convenienza".

Mettendo insieme le poche informazioni ottenute finora, possiamo osservare che per quanto riguarda la riconciliazione, i sifaka sono simili a *Eulemur fulvus* mentre si differenziano da *Lemur catta* e *Eulemur macaco*, che hanno società più "rigide", con livelli bassissimi o nulli di riconciliazione. Un'altra conclusione che si può trarre dallo studio, dunque, è che la capacità di ripristinare buoni rapporti dopo una aggressione sembra essere più legata ai livelli di tolleranza all'interno della società che non alla vicinanza filogenetica, giacché le specie di *Eulemur* e *Lemur catta* (famiglia Lemuridae) sono molto più imparentate tra loro che con il sifaka (famiglia Indridae).

È dunque possibile vivere in gruppo senza avere comportamenti riconciliatori? In alcuni casi sì, ma, di solito, non a lungo termine. L'evoluzione ha "scelto" due vie per preservare la stabilità di un gruppo sociale: quella del despotismo, che nelle società umane è paragonabile a una dittatura o oligarchia, e l'egalitarismo, che potremmo associare, per analogia, ai sistemi democratici. Nelle società "tiranniche" il despota regola e controlla le relazioni tra gli individui, fungendo da "collante" sociale. In questo modo, vi è poco spazio per una risoluzione autonoma dei conflitti tramite contatti affiliativi. Esistono anche meccanismi di riduzione della tensione e dello stress che, a monte, evitano l'insorgenza di conflitti. Tuttavia, quando c'è un ribaltamento gerarchico, il gruppo può andare incontro alla disgregazione. Nelle società egalitarie gli individui sono più liberi di interagire, anche quando esiste una gerarchia ben definita e lineare, come nel caso del sifaka. È in queste società che si riscontra la massima capacità di mantenere la stabilità sociale. Tale capacità arriva, evidentemente, da lontano. ●

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S’accoupler d’abord, s’accoupler plusieurs fois : les fluctuations du marché biologique dans des prosimiens.

Introduction:

En biologie, en économie et en politique, le pouvoir distributif est la clé pour comprendre les relations asymétriques entre individus qui peuvent être obtenues par la force (dominance) ou la négociation (effet de levier). Chaque fois que les mâles ne peuvent pas recourir à la force, ils dépendent en grande partie des femmes pour la possibilité de se reproduire ; pour cela, la balance du pouvoir penche en faveur des femmes. Ainsi, les mâles non seulement rivalise au sein de leur classe de sexe (mâles avec mâles), mais aussi échange des services avec le sexe opposé. Ce marché de l'accouplement, décrit pour les humains et les singes, peut-il être appliqué aussi aux prosimiens, les primates le plus ancestraux? Pour répondre à cette question, nous avons étudié dans la dominance le lémurien grégaire, *Propithecus verreauxi* (sifaka), caractérisé par la dominance des femelles sur les mâles, un accouplement promiscue et une reproduction saisonnière.

Résultats et Discussion

Nous avons observé 57 évents de copulation impliquant 8 mâles et 4 femelles dans la réserve de Berenty (Sud Madagascar), et toutes les événement sur le toilettage, les agressions, et le comportement de marquage. Nous avons effectué des analyses via Spearman (test exacte) et des matrices de corrélations. L’ordre avec qui les mâles arrivaient à s’accoupler avec les femelles était en corrélation avec la fréquence avec laquelle les mâles marquaient sur les marquages effectués par les femelles (sur-marquage), mais il n’était pas en corrélation avec la proportion de combats gagnés par les mâles. Ainsi, la compétition des mâles est de type olfactif et n’est pas basée sur les agressions. La fréquence de copulation n’était pas corrélée avec la proportion de combats gagnées par les mâles et n’était pas corrélée avec la fréquence de sur-marquage. Par contre, pendant la période des accouplements, la fréquence de copulation était corrélée avec la fréquence de toilettage des mâles vers les femelles. Dehors de cette période, la fréquence de toilettage des mâles vers les femelles était corrélée avec la fréquence de toilettage des femelles vers les mâles. En bref, le marché biologique est sujet à des fluctuations saisonnières, car les mâles ont négocié le toilettage pour le sexe dans la période d'accouplement et le toilettage pour le toilettage dans la période précédant l'accouplement.

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Mating First, Mating More: Biological Market Fluctuation in a Wild Prosimian

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Abstract

In biology, economics, and politics, distributive power is the key for understanding asymmetrical relationships and it can be obtained by force (dominance) or trading (leverage). Whenever males cannot use force, they largely depend on females for breeding opportunities and the balance of power tilts in favour of females. Thus, males are expected not only to compete within their sex-class but also to exchange services with the opposite sex. Does this mating market, described for humans and apes, apply also to prosimians, the most ancestral primate group? To answer the question, we studied a scent-oriented and gregarious lemur, *Propithecus verreauxi* (sifaka), showing female dominance, promiscuous mating, and seasonal breeding. We collected 57 copulations involving 8 males and 4 females in the wild (Berenty Reserve, South Madagascar), and data (all occurrences) on grooming, aggressions, and marking behaviour. We performed the analyses via exact Spearman and matrix correlations. Male mating priority rank correlated with the frequency of male countermarking over female scents but not with the proportion of fights won by males over females. Thus, males competed in an olfactory tournament more than in an arena of aggressive encounters. The copulation frequency correlated neither with the proportion of fights won by males nor with the frequency of male countermarking on female scents. Male-to-female grooming correlated with female-to-male grooming only during premating. Instead, in the mating period male-to-female grooming correlated with the copulation frequency. In short, the biological market underwent seasonal fluctuations, since males bargained grooming for sex in the mating days and grooming for itself in the premating period. Top scent-releasers gained mating priority (they mated first) and top groomers ensured a higher number of renewed copulations (they mated more). In conclusion, males maximize their reproduction probability by adopting a double tactic and by following market fluctuations.

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Introduction

In biology, as well as in economics and politics, power is a key concept for understanding asymmetrical dyadic relationships [1]. Distributive power [2] can originate from both dominance (when force is used) and leverage (when the use of force is not possible). An individual has leverage over another when that individual possesses something that the other needs but cannot acquire through coercion [3]. In this case, trading becomes essential for mutually beneficial interactions within social groups, both in economical and biological markets [4]. An important feature of market models is that the expected future gains are actively influenced by playing off potential partners against each other [5,6]. The typical game theory approach includes only two players and, although this is changing within economics as well as biology, the classical models do not take into account partner choice [4]. In contrast, the biological market theory includes multi-player models, that is theoretical games with at least three or more "players" (traders, in the market systems) [7]. Two or more classes of traders (sex classes, rank classes, etc.) exchange commodities in biological markets to their mutual benefit. Different group members can offer different kinds of commodities in exchange for alternative ones that they do not currently possess [4]. Usually, competition acts as the driving force within the same trader class (including all members offering the same kind of commodity) while cooperation can occur between different trader classes [4,8].

In the mating market, the balance of power tilts in favour of females whenever males cannot force females into mating (as it happens in sexually monomorphic species or when females form coalitions) [3]. Consequently, males depend on females for breeding opportunities and must compete to prove their superiority to females, thus increasing their possibility to be selected [3,9]. Males can engage in both contest competition via physical/ritualized fighting and outbidding competition, in which a male plays off rivals by making a better offer [4]. In the latter case, males can secure the favours of a female by advertising their quality (e.g. the dominance status) through visual or olfactory displays [10,11] and/or by being more generous than others in providing a commodity in exchange for female access (competitive altruism) [8,12]. One of the most valuable commodity that can be offered in social mammal groups is grooming, which is used for parasite removal [13], stress reduction [14], and as social cement to start, consolidate, or repair relationships [15]. Grooming is a commodity that can be exchanged for itself or for breeding opportunities [16].

Sociality is widespread among mammals [17] and particularly among anthropoid primates (monkeys and apes [18]). In prosimians (the most ancestral group of primates) sociality is the exception more than the rule. Among Malagasy prosimians (lemurs), few species combine a powerful olfactory system (retained from basal mammals) and puzzling features like group living, female priority over resources, and absence of sexual dimorphism [19]. Such combination of features makes gregarious lemurs the ideal model to understand the biological bases of mate selection by females, who cannot be accessed by force or using food as exchange commodity. In particular, we selected the diurnal species *Propithecus verreauxi* of south/southwest Madagascar [20] to find out which male strategies are successful to maximize breeding opportunities (Figure 1).

Results

The rank of mating priority assigned to males did not correlate with their mating frequency (Exact Spearman $r_s = -0.434$, $n = 8$, $p = 0.284$; $\alpha = 0.01$ adjusted via Bonferroni).

Male priority rank correlated with the frequency of male countermarking (Exact Spearman $r_s = 0.866$, $n = 8$, $p = 0.005$; $\alpha = 0.01$) but did not correlate with i) the proportion of fights won by males in presence of females (Exact Spearman $r_s = 0.448$, $n = 8$, $p = 0.265$; $\alpha = 0.01$) and ii) the frequency of grooming directed by males to females (Exact Spearman, $r_s = -0.099$, $n = 8$, $p = 0.816$; $\alpha = 0.01$) and by females to males (Exact Spearman, $r_s = 0.138$, $n = 8$, $p = 0.744$; $\alpha = 0.01$).

The mating frequency correlated neither with the proportion of fights won by males in presence of females ($Kr = 22$, $\tau_{Kr} = 0.284$, $P = 0.057$, $\alpha = 0.0125$ adjusted via Bonferroni) nor with the frequency of male countermarking on female depositions ($Kr = 16$, $\tau_{Kr} = 0.209$, $P = 0.103$). In the breeding period, mating frequency correlated with the frequency of grooming directed by males to females (MF grooming; $Kr = 26$, $\tau_{Kr} = 0.609$, $P = 0.001$, $\alpha = 0.0125$ adjusted via Bonferroni) but not with the frequency of grooming performed by females to males (FM grooming; $Kr = 12$, $\tau_{Kr} = 0.336$, $P = 0.091$).

MF grooming and FM grooming correlated in the premating period ($Kr = 28$, $\tau_{Kr} = 0.675$, $P < 0.001$, $\alpha = 0.0125$ adjusted via Bonferroni) but not in the mating days ($Kr = 3$, $\tau_{Kr} = 0.157$, $P = 0.282$). FM grooming significantly decreased in the mating days compared to the premating period (Wilcoxon Signed Ranks Test $T = 0$, $P = 0.008$, $n = 8$) while MF grooming did not differ between the two periods (Wilcoxon Signed Ranks Test $T = 6$, $P = 0.102$, $n = 8$) (Figure 2).

Discussion

Whenever individuals cannot forcibly appropriate valuable resources without the consent of the owner, they should compete for partners and negotiate about resource distribution in biological markets [4]. Specifically, the mating market should involve a) male-male competition to gain female access and b) male-female trade, in which males bargain services for breeding opportunities [21,22,23].

Male-male competition for female access

Aggressive interactions are a widespread form of competitive strategy adopted by males to gain female access [24]. However, in the sifaka we found no correlation between the proportion of fights won by each male and mating frequency and priority. Since the sifaka society is characterized by female dominance and philopatry [25], it is not surprising that male fighting ability is unimportant in

Figure 1. Sifaka copulation: picture taken during a mating episode. Photo by Daniela Antonacci *via* Panasonic Lumix DMC FZ7 - 12× optical zoom/36–432 mm equivalent/LEICA lens.
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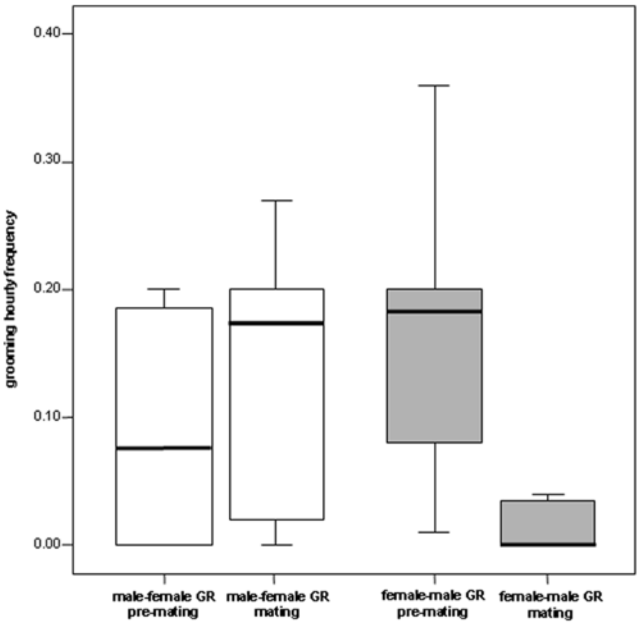

Figure 2. Grooming interchange. Variation in the levels of grooming directed from males to females and from females to males in the two periods (premating and mating).
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female mate choice. In general, winning a fight does not necessarily confer sexual access on males. In fact, females can base their mate choice on other features (e.g., age, time spent in the group, male physiological status, etc.) especially in those species in which females can acquire a dominant or co-dominant status, individually or by forming coalitions [26,27,28].

In scent oriented species, male competition for females can be translated into an olfactory tournament (outbidding competition) more than into an arena of aggressive encounters (contest competition) [9,11,29,30,31]. Scent marks provide a reliable signal of competitive ability [29,28,32]. Sifaka males competed for females by countermarking female odour depositions: in the end, the most active males gained breeding priority (Video S1). As a matter of fact, sifaka males can use scent marking as a form of self-advertisement for mating purposes [33] possibly because odour signals convey information on dominance status, which is one of the main choice criteria adopted by females [24,34]. The importance of olfactory male competition in female mate choice has been provided for non primate species [29] as well as for primate ones, including New World monkeys [35] and prosimians. In particular, females of *Nycticebus pygmaeus* (a nocturnal prosimian) rely on olfactory deposition frequency to select mating partners [36]. Moreover, during the premating period *Lemur catta* males compete for female access via ritualized "stink fights" and females increase their tolerance towards males based on the outcome of such fights [37,38].

Male-female *do ut des* for breeding opportunities

In the sifaka, the higher mating priority gained by males via scent marking activity did not match with a higher number of copulations. In fact, mating first does not necessarily mean mating more. In order to increase their breeding opportunities, males had to move from theory to facts, by offering a service in exchange for sex (mating market) after a self-promotion phase via odour messages.

Grooming and food are the two main valuable commodities that a male can offer to a female [24,39,40,41,42]. However, food is not a spendable commodity in the sifaka society, since females have unquestioned feeding priority [43]. We found that in the premating period grooming performed by males to females positively correlated with grooming performed by females to males (grooming reciprocity). Instead, in the mating period we found that grooming performed by males to females was correlated with the frequency of copulations but not with grooming received by males from females (Video S2). These results indicate that grooming was traded for itself in the premating period (interchange) and for mating opportunities in the mating period (exchange). In short, males used the same commodity across the study period, whereas females switched from grooming to breeding availability during the mating period.

Grooming market has been found also in other primate species. Barrett and Henzi [39] found that in chacma baboons (*Papio cynocephalus ursinus*) grooming exchanged within females was affected by the rank distance between individuals. Similarly, Port et al. [44] found that in the redfronted lemur (*Eulemur fulvus rufus*) grooming trade was influenced by rank position. In fact, subordinates traded grooming for itself with other subordinates and for social tolerance with dominants [44]. The fact that sifaka females can mate also with out-group individuals [45] indicates that mate choice by females goes beyond the relative ranking status within males belonging to a stable foraging group. Yet, by chest condition (stained; Palagi et al., unpublished data), we can infer that out-group males were probably high ranking individuals in their groups of origin.

The exchange of grooming for sexual access is not uncommon even in societies characterized by male dominance [39,46]. In fact, regardless of the dominant sex, the leverage of females increases when they are in oestrous because they have an inalienable commodity: their eggs ready to be fertilized [3]. For example, male baboons use grooming to ensure females tolerate them in close proximity so that they can exclude other males and achieve a high frequency of mating [39,47]. In chimpanzees, low ranking males need to provide more grooming to oestrus females than high ranking males in order to gain female access [42]. Beyond primates, Stopka and MacDonald [40] found that females of *Apodemus sylvaticus* (a mouse species characterized by promiscuous mating system without any paternal investment) require grooming before allowing a male to progress towards sex. The same authors hypothesized that females could obtain grooming through a process of "unintentional bargaining" [*sensu* 40]: in such species, grooming was the only commodity which males had been seen to provide in the process of mate selection.

In conclusion, mate choice by sifaka females is complex and a single factor cannot explain it all. Many males can compete and occasionally obtain female access but only top scent-releasers and groomers reach the highest mating priority and rates, thus maximizing their reproduction probability. On a broader perspective, we demonstrated not only that the biological market paradigm can successfully be applied to prosimians but also that such market undergoes seasonal fluctuations, shifting from a grooming to a mating market over time.

Materials and Methods

Study species and site

We conducted this study in the secondary forest of Ankoba, in the Berenty Reserve (South Madagascar; S 24.99°; E 46.29°; for an extensive description see [48]) on *Propithecus verreauxi* (Verreaux' sifaka).

Table 1. Descriptive statistics of the sifaka counted and sexed at Berenty in 2006: total number of groups and individuals, number of adult males and females, and number of infants; minimum, maximum and standard deviation (STD) of the number of individuals (of both sexes), males, females, and infants per group [50].

	Total number	Min/ Group	Max/ Group	Mean	STD
Groups	49	1	10	4.22	2.16
All animals (infants and adults)	229	1	10	4.67	2.40
Adult Males	127	0	7	2.59	1.62
Adult Females	79	0	4	1.61	0.89
Infants	23	0	2	0.47	0.62

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The sifaka are social and diurnal prosimians that live in relatively stable groups (spanning 2–13 individuals, e.g. at Beza-Mahafaly, South West Madagascar [49] and Kirindy, West Madagascar [33]). At Berenty, sifaka groups range from 1 to 10 individuals, according to a complete census conducted in November-December 2006 [50]. As with other lemur species, sifaka groups are characterized by an unbalanced sex-ratio, which is skewed towards males [19,50,51] (Table 1).

They inhabit riverine and dry forests of south and southwest Madagascar [20] and are sexually monomorphic (or females are larger than males; [52]). Moreover, the sifaka are characterized by female philopatry and social dominance and by the absence of male infant care [20]. Sifaka males are very active in scent marking via both sternal glands (abortive in females) and ano-genital secretions [37]. Moreover, sifaka males are bimorphic in chest status: the ones that are most active in scent marking show a pronounced brown staining around their sternal gland (stained chested males) while the others do not (clean chested males) [34]. Stained-chested males (different from clean-chested males) usually occupy a dominant position in sifaka groups [34]. Females usually experience a single oestrus period (2–3 days) per year and both sexes can mate with multiple partners in their own and neighbouring groups, especially when a single group offers suboptimal mating opportunities [53]. In particular, males can start roaming and visiting other groups in search of oestrus females [45]. The short oestrus period and the fact that mating can be tightly synchronized within a population make copulations very difficult to detect and observe [25,34]. Moreover, at Berenty, cyclones and heavy raining followed by river flooding normally prevent data collection in the period January-February, coinciding with sifaka's mating period. In 2007, for the first time it was possible to gather data on mating because of a prolonged drought involving South Madagascar. In the end, we gathered the highest sample of mating episodes ever recorded in prosimians.

Observational data and operational definitions

Mating, observed in one group, involved in-group members (6 males and 4 females) and 2 out-group males both showing a stained chest (all animals were individually identified according to their external features, [37]). Group composition and sex-ratio were typical for the study species in general [49] and for the study population in particular [50] (cf. Table 1). As reported at Beza-Mahafaly [45], also at Berenty males started visiting neighbouring groups prior to the mating days. As a matter of fact, several out-group males started visiting our study group 23 days before the first mating day. We were able to collect standardized data on two of them, which visited and spent 70% of time with the study group. It was not possible to pool out-group with resident males to draw a dominance hierarchy because the time spent by out-group males with residents was not enough to allow any statistical analysis in this respect.

The premating period was defined as the month prior to the mating days. The authors and a field assistant collected mating, grooming, aggressive interactions, and scent marks via all-occurrences (221 hr; [54]), during daily continuous observations (about 11 h/day) on both in-group and out-group members. Data were collected from December (2006) to February (2007) when the observations had to be stopped because of storming weather.

We collected 53 male-male aggressions, 551 male marking bouts, and 72 allo-grooming bouts. As typical of the sifaka the individuals of the group usually moved, rested, and foraged cohesively. However, the group could split during the mating days: in this case, the observers separated to follow the two different subgroups.

Brockman, who observed sifaka mating in a different study site (Beza-Mahafaly; Southeastern Madagascar; [22]), provided the operational definitions used during this study. In particular, mating referred to copulatory behaviour in which intromission and thrusting were unambiguously observed (Figure S1 and Video S3). During our study, copulations lasted from 11 sec to 7 min ($N = 57$, mean: 1.860 min±1.603 SE). Mount occurred for less than 3 sec without intromission and thrusting, and were usually associated with female resistance. Ejaculation, generally not visible, was inferred based on a rapid increase in thrusts and a pause just prior to the dismount, followed by intense genital self-grooming [45,55]. In this study, only proper copulations were included in the analysis.

To calculate the mating priority index we first ranked males according to the order by which they accessed each oestrus female (male priority rank). When a male did not access to one oestrus female at all, the rank assigned to the male for that female was 0. Then, the rank sum for each male was averaged on the number of oestrus females. The male priority rank has not to be confounded with the hierarchical position of males within their own groups (dominance ranking position).

Statistical analyses

The analyses were conducted at dyadic and individual level ($N_{males} = 8$; $N_{females} = 4$). Behavioural bouts per individual (mating episodes, aggressions, grooming, and scent marks) were normalized on the observation time (hours).

We used the Rowise Matrix Correlation test using rectangular matrices (MatrixTester 2.2.2b by Hemelrijk 2001) to verify the relationship between mating frequency and a) the proportion of fights won by males in presence of females, b) male counter-marking on female depositions c) male-to-female and female-to-male grooming. With the same method we also tested the correlation between female-to-male and male-to-female grooming during the mating and premating days.

Due to the small sample size and deviation from normality (Kolmogorov-Smirnov<0.05) we used non parametric statistics (software: Statxact 8, Cytel Studio, and SPSS 12.0). In particular we adopted the Spearman test to correlate the rank of mating priority with the frequency of a) mating episodes; b) male countermarking on female depositions; c) fights won by males in presence of females; d) male-to-female and female-to-male grooming. Moreover we used the Wilcoxon match-pairs signed rank test to compare the frequency of male-to-female and female-to-male grooming between premating and mating days.

Exact values were applied following [56] and, when needed, the significance level ($\alpha = 0.05$) was adjusted downward following the Bonferroni technique [57].

Supporting Information

Figure S1 Details of a copulation (photo by Daniela Antonacci via Panasonic Lumix DMC FZ7 - 12× optical zoom/36–432 mm equivalent/Leica Lens)
Found at: doi:10.1371/journal.pone.0004679.s001 (3.21 MB TIF)

Video S1 Male countermarking behaviour on a female scent deposition (video by Daniela Antonacci via Canon DM MV 600-18× optical zoom/2.8–50 mm equivalent/Canon Video Lens).
Found at: doi:10.1371/journal.pone.0004679.s002 (10.01 MB MOV)

Video S2 Copulation followed by a grooming session (video by Daniela Antonacci via Canon DM MV 600-18× optical zoom/2.8–50 mm equivalent/Canon Video Lens).
Found at: doi:10.1371/journal.pone.0004679.s003 (10.21 MB MOV)

Video S3 Copulation in which intromission and thrusting were unambiguously observed (video by Daniela Antonacci via Canon DM MV 600-18× optical zoom/2.8–50 mm equivalent/Canon Video Lens).
Found at: doi:10.1371/journal.pone.0004679.s004 (9.99 MB MOV)

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Author Contributions

Conceived and designed the experiments: IN EP. Performed the experiments: IN DA EP. Analyzed the data: IN DA EP. Wrote the paper: IN EP.

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Sesso, politica... e proscimmie

Daniela Antonacci

Già nei lemuri, i primati più antichi, la concessione di «favori sessuali» segue le regole di mercato

di Ivan Norscia, Daniela Antonacci ed Elisabetta Palagi

“L’inclinazione a trafficare, barattare, scambiare una cosa con un’altra è comune a tutti gli uomini e non si trova in nessun’altra razza di animali», sosteneva nel 1700 Adam Smith, l’economista e filosofo scozzese considerato il fondatore dell’economia politica.

In realtà il *do ut des* ha radici ben più antiche della civiltà umana, dal momento che lo scambio di servizi è più antico dell’uomo, delle antropomorfe e delle scimmie stesse. Il nostro gruppo di ricerca ha infatti recentemente dimostrato che già nelle proscimmie, il gruppo più ancestrale di primati, è presente una politica economica basata sullo scambio di benefici.

La contaminazione tra economia e biologia si è rivelata proficua fin dai tempi di Darwin, che plasmò la sua teoria dell’evoluzione per selezione naturale traendo spunto anche dalle teorie economiche di Malthus. In maniera analoga, la teoria del mercato biologico (*biological market*) prende spunto da quella economica della domanda e dell’offerta.

In biologia, così come in economia e politica, il potere è un concetto chiave per capire le diverse relazioni tra gli individui. Esso può essere fisico, quando un individuo può surclassare un altro con la forza, o economico, quando un individuo detiene risorse ambite che può offrire a un altro in cambio di un equo «compenso». Il potere economico si manifesta quando un individuo possiede una risorsa che l’altro desidera, ma che non può ottenere tramite coercizione fisica. In questo caso la capacità di mercanteggiare e scambiare beni e servizi diventa cruciale per le relazioni che costituiscono il fondamento primo dei gruppi sociali.

GIOCHI O MERCATI?	TEORIA DEI GIOCHI CLASSICA	TEORIA DEI MERCATI BIOLOGICI
Numero di parti in gioco	2	> 2
Tipi di parti in gioco	2 giocatori contrapposti	Almeno 2 classi di giocatori contrapposte
Meccanismi di gioco	Competizione tra le due parti	Competizione intra-classe Cooperazione inter-classe
Scelta del partner	Un partner alla volta se il gioco è reiterato ♂ ♀	Scelta tra più partner ♂ ♂ ♂ ♂ ♀ ♂ ♂

LA CONTAMINAZIONE TRA ECONOMIA e biologia si rivela proficua fin dai tempi di Darwin. A fianco, uno schema di confronto tra la classica teoria dei giochi e quella dei mercati biologici, che prende spunto dalla teoria economica della domanda e dell'offerta.

Giochi e mercati... in teoria

La classica teoria dei giochi prevede una «sfida» o competizione tra due parti che hanno interessi contrapposti e che potranno reiterare il gioco con partner diversi (ne sono un esempio i modelli basati sul dilemma ripetuto del prigioniero). Secondo questo principio, un maschio si trova ad «affrontare» una femmina alla volta per garantirsi la possibilità di riprodursi. Alla fine della partita, la femmina o il maschio potranno cambiare «avversario» e iniziare un nuovo «gioco» (per esempio un nuovo rituale di corteggiamento). Benché maschi e femmine in apparenza perseguano lo stesso scopo (riprodursi), i due sessi sono perennemente in conflitto: il maschio ha tutto l'interesse a disseminare i propri spermatozoi, mentre la femmina ha tutto l'interesse a non sprecare il proprio uovo, selezionando fortemente il maschio. In sostanza, mentre il maschio punta sulla quantità, la femmina punta sulla qualità. Questa dicotomia trae origine dal fatto che mentre il numero di ovuli è già determinato alla nascita, quello degli spermatozoi (gameti a basso costo energetico) non lo è.

La teoria del mercato biologico non prevede più due giocatori, ma almeno tre, che potremmo chiamare «venditori». I venditori appartengono almeno a due classi diverse. Tra gli individui della stessa classe scatta una competizione per accedere a un beneficio messo «in palio» dal venditore (o venditori) della classe opposta. I maschi apparterranno quindi alla stessa classe perché forniscono uno stesso servizio, e competeranno tra loro per accaparrarsi il beneficio offerto da una o più femmine, cioè l'opportunità di riprodursi. Per ottenere l'accesso alla risorsa, i maschi dovranno instaurare un rapporto di cooperazione con le femmine, offrendo dei «servigi». Il miglior offerente verrà scelto per l'accoppiamento, e i criteri di scelta delle femmine potranno variare in funzione dell'ecologia e della sociobiologia della specie.

Ivan Norscia

Nei mercati, la possibilità di accaparrarsi guadagni futuri è, dunque, subordinata alla capacità di sgominare potenziali competitori in una piazza dove più individui «concorrono» per poter accedere a una risorsa ambita o a un privilegio. *Conditio sine qua non* affinché questo meccanismo possa realizzarsi è che la risorsa sia limitata e non monopolizzabile; non condivisibile da più competitori. Un cibo prelibato, una femmina in estro, una cattedra accademica, una poltrona in Parlamento, tanto per citare alcuni esempi. In tale piazza, la promozione è un passo cruciale verso il successo, ma non l'unico. Infatti una volta acquisito l'accesso privilegiato alla risorsa è necessario mantenerlo nel tempo, e ciò è possibile soltanto in un modo: dimostrando in pratica quanto pubblicizzato in precedenza.

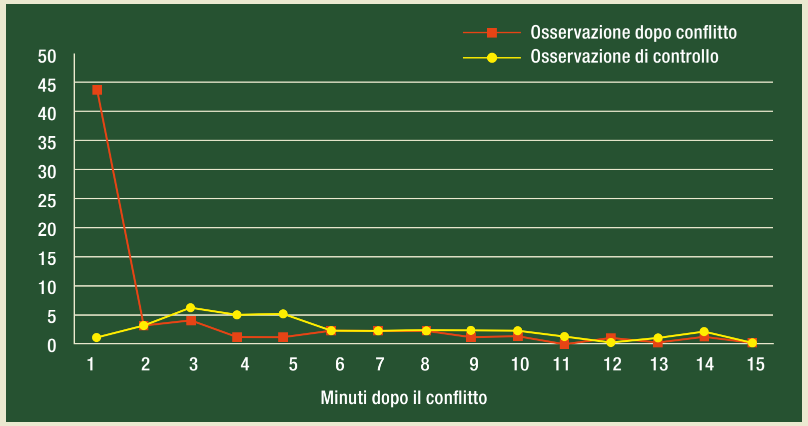
Questo fenomeno, prima che economico, politico e sociale, è biologico. Un caso particolare di mercato biologico è quello che scatta nel periodo degli accoppiamenti, che in molte specie animali è incredibilmente breve. In questo «momento storico» le relazioni tra maschi e femmine cambiano radicalmente, perché le femmine detengono una risorsa estremamente appetibile per i maschi: l'ovulo pronto per essere fecondato. Le loro quotazioni svettano, soprattutto se i maschi non possono impossessarsi con la forza della risorsa in gioco. Questo è il caso dei lemuri.

Competizione?... Non solo!

Se la competizione è un elemento regolante e regolatore della vita sociale, come mai molte specie animali costituiscono gruppi permanenti e ben strutturati? Perché non è l'unico.

Esistono sistemi comportamentali per aumentare i livelli di tolleranza e riparare relazioni interrotte o dissipare la tensione causata da conflitti all'interno del gruppo sociale. Fare la pace, riconciliare, è uno di questi meccanismi naturali. Negli anni ottanta Frans de Waal, pioniere degli studi sul comportamento post-conflittuale, ha messo a punto un metodo per misurare in modo oggettivo la capacità di «fare pace» degli animali, tarandolo su scimpanzè in cattività. Mentre la riconciliazione è stata ampiamente investigata nei primati «superiori», poche sono le informazioni provenienti dal mondo delle proscimmie.

In uno studio recente, svolto a Berenty, abbiamo dimostrato che la riconciliazione è presente anche nei sifaka in natura. L'aspetto più interessante è che queste proscimmie sono in grado di valutare rischi e benefici derivanti da un riavvicinamento con l'aggressore. Infatti le vittime sono più inclini a riconciliare dopo scontri di lieve intensità, perché il rischio è minore, e con le femmine più alte in gerarchia, perché il beneficio è maggiore. In accordo con l'ipotesi delle buone relazioni (*Good Relationship Hypothesis*), si riconcilia di più tra individui che condividono buoni rapporti sociali, determinati in base alla quantità di grooming scambiato.



Daniela Antonacci

Lemuri e mercato biologico

Ogni volta che i maschi non possono esercitare coercizione fisica sulle femmine, la bilancia del potere pende a favore di queste ultime: sono le femmine, insomma, il vero sesso forte. Questa situazione si verifica quando esse formano coalizioni (come accade per esempio nei bonobo, *Pan paniscus*) o hanno «doti fisiche» paragonabili a quelle dell'altro sesso.

Nelle proscimmie il dimorfismo sessuale è assente: maschi e femmine hanno dimensioni paragonabili. La dominanza femminile può andare dalla semplice priorità di accesso alle risorse, come accade spesso nei sifaka (*Propithecus verreauxi*), fino all'attuazione di strategie fortemente bellicose, come nel caso tipico del lemure dalla coda ad anelli (*Lemur catta*).

La difficoltà di osservare le proscimmie, riconoscere gli individui e quantificarne i comportamenti deriva dal fatto che la maggior parte di esse con-

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Parchi zoologici per la conservazione

Il sifaka (*Propithecus verreauxi*) è una specie diffusa nel Madagascar meridionale e occidentale, ed è tipica di foreste secche (decidue e spinose) e fluviali. Gran parte del suo areale coincide con la foresta spinosa del sud del Madagascar, classificata tra le 200 regioni più importanti del mondo dal punto di vista ecologico. Il 48 per cento dei generi e oltre il 95 per cento delle specie vegetali che compongono la foresta sono, infatti, endemiche. I sifaka, che vivono in gruppi di 3-11 individui, sono molto difficili da preservare *ex situ*: solo pochi individui sono presenti in parchi-zoo al di fuori del Madagascar. La difficoltà sta nel fatto che questi lemuri combinano una locomozione dispendiosa dal punto di vista energetico (salto e arrampicata verticale) a una dieta estremamente povera di zuccheri e proteine, prevalentemente costituita da foglie adulte. In gergo primatologico si parla, infatti, di animali «foglivori» (una particolare categoria di erbivori). La foresta spinosa, poi, rappresenta di per sé un ambiente ostile e ostico, con un terreno arido e risorse alimentari scarse.

È alla luce di questa situazione che tre parchi-zoo italiani, Pistoia, Falconara e Lignano Sabbiadoro, hanno supportato il nostro gruppo di ricerca nel progetto di conservazione e salvaguardia *in situ* attraverso una campagna di censimento della popolazione di sifaka che vive nelle foreste dell'area di Berenty (Madagascar meridionale). Secondo i nostri dati, i sifaka che vivono nelle zone frammentate di foresta spinosa sono in numero ridotto, ma presentano una *sex-ratio* (la proporzione di maschi e femmine nella popolazione) più bilanciata rispetto a quelli che vivono nelle aree «non spinose» della riserva Berenty. In realtà, rispetto alla popolazione della foresta spinosa, quella della riserva presenta un maggior sbilanciamento: oltre i due terzi sono maschi. Questo sbilanciamento è preoccupante, perché riflette una condizione di stress dovuta al drastico calo della risorsa idrica.

duce vita notturna e pressoché solitaria. I sifaka invece hanno caratteristiche che li avvicinano più alle scimmie che alle proscimmie «tipiche». Si tratta infatti di animali diurni, che vivono in gruppi sociali più o meno numerosi. Gli individui formano una società strutturata a gerarchia lineare, di tipo non dispotico, caratterizzata da intensi scambi di contatti sociali. La socialità è un prerequisito fondamentale per gli studi etologici che, come il nostro, si occupano di mercati biologici.

Per lo studio abbiamo osservato i sifaka della foresta galleria di Berenty (nel Madagascar meridionale) durante la stagione umida. Il nostro scopo era valutare se e come le femmine scegliessero i loro partner sessuali. Domande lecite per una specie in cui la promiscuità la fa da padrona. In particolare eravamo interessati ai servizi offerti dai maschi per «sedurre» le femmine e garantirsi maggiori opportunità riproduttive.

Il *grooming* (pulizia del pelo di un conspecifico effettuata con le mani o con la bocca) è una delle più ambite monete di scambio nei primati non umani: non solo ha funzioni igieniche, ma agisce da vero e proprio collante sociale, permettendo di instaurare e consolidare relazioni amichevoli con gli altri individui. La presenza di un mercato del *grooming* nelle proscimmie è stata suggerita solo di recente. Tuttavia l'effettiva presenza di un mercato e delle sue fluttuazioni nel tempo non è mai stata dimostrata nei primati più antichi.

La «campagna elettorale» dei maschi

In generale, nella stagione degli accoppiamenti tra i maschi scatta una vera e propria «corsa all'ovulo», che può avvenire tramite incontri/scontri fisici oppure tattiche di gioco più sofisticate, volte a escludere gli avversari dalla competizione.

Ivan Norscia (3)

Nelle società a dominanza o co-dominanza femminile, il duello tra maschi non è un mezzo utile per conquistare la preferenza delle femmine, che basano le loro scelte su altri parametri come l'età (elefanti), il tempo trascorso nel gruppo di appartenenza (iene maculate) o lo stato di salute (molte specie di roditori).

Anche nei sifaka i maschi si giocano la possibilità di accedere per primi alle femmine non in un'arena di scontri aggressivi, ma attraverso una campagna elettorale che si svolge a colpi di slogan odorosi. Perché, tra le diverse forme di competizione, proprio quella odorosa è la più efficace? Le proscimmie presentano speciali adattamenti sia per il rilascio del segnale odoroso che per la sua ricezione, adattamenti ridotti o persi nelle scimmie che affidano invece ai segnali visivi gran parte della loro comunicazione sociale. I polsi, la gola, la coda, gli organi genitali sono strumenti di cui i lemuri si avvalgono per diffondere, cospargere, spruzzare le molecole odorose (feromoni) che regolano i rapporti sociali.

Le marcature odorose sono biglietti da visita attendibili, dato che gli odori sono segnali necessariamente «onesti» (cioè non camuffabili o alterabili) perché legati intimamente alla fisiologia dell'animale. L'odore fornisce informazioni sulla qualità del potenziale partner e in particolare sul grado di dominanza e lo stato di salute del maschio.

Gli sforzi che i maschi fanno per disseminare il territorio di segnali odorosi sono ripagati dalle femmine con un accesso privilegiato all'accoppiamento, accesso che non viene garantito ai maschi che, invece, escono vittoriosi da un maggior numero di scontri. I nostri dati indicano chiaramente che i maschi più attivi nella campagna odorosa sono proprio quelli che si aggiudicano le prime copule.

Dalla teoria alla pratica: mantenere le promesse elettorali

Accoppiarsi per primi non vuol dire necessariamente accoppiarsi di più. Per mantenere il privilegio acquisito occorre passare dalla teoria ai fatti. Dopo la prima elezione, per essere «votati» nuovamente, i maschi devono mantenere le promesse elettorali, tanto per continuare a usare una metafora politica. I nostri risultati evidenziano infatti che, per aggiudicarsi un maggior numero di copule, i vincitori delle «primarie» devono offrire ed effettuare un servizio concreto: il grooming.

Nei sifaka il mercato del grooming è aperto e attivo tutto l'anno. Al di fuori della stagione degli amori, il servizio di pulizia del pelo è scambiato alla pari tra i due sessi; abbiamo infatti rilevato una correlazione positiva tra il grooming effettuato e quello ricevuto nelle diadi maschio-femmina (*interchange*). Questa correlazione salta nel periodo degli amori, quando le femmine ripagano il grooming dei maschi con un altro tipo di valuta: l'opportunità di riprodursi (*exchange*). La correlazione positiva riguarda in questo caso le frequenze di grooming effettuato dai maschi e le loro frequenze di accoppiamento. In sostanza, chi è più attivo nella pulizia del pelo si aggiudica il maggior numero di copule.

Per concludere, i sifaka maschi massimizzano la loro probabilità di successo riproduttivo attraverso una duplice strategia. Molti maschi possono competere tra loro e ottenere, occasionalmente, l'accesso alle femmine, ma solo quelli più attivi nelle marcature odorose e nel grooming arrivano ad accoppiarsi prima e più spesso.

Allargando questa prospettiva, con il nostro studio abbiamo dimostrato non solo che lo scambio di servizi è applicabile anche alle proscimmie, il gruppo più antico di primati, ma anche che questo scambio segue le fluttuazioni stagionali del mercato. In termini evolutivi, il mercato «sessuale», dunque, potrebbe risalire a più di 50 milioni di anni fa, quando avvenne la separazione delle proscimmie dall'antenato in comune con gli umani e le altre scimmie, antropomorfe e non antropomorfe. ■

➥ Letture

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American Journal of Physical Anthropology (2009) 139 : 182-192

Signalisation multimodal dans *Lemur catta* en liberté: économie du signal et fonction territoriale du marquage avec l’urine.

Introduction

Le marquage urinaire a été négligé dans les prosimiens. Des études ont montré que les lémuriens Malgaches en captivité effectuent un marquage odoré en utilisant l’urine, ainsi que par l'intermédiaire des secrétions spécialisés. *L. catta* utilise l’urine pour marquer et cela représente un signal multimodal qui diffère de la simple miction en termes de différentes caractéristiques de conception, y compris la configuration de queue: la queue est maintenue en haut pendant le marquage (UT-up) et vers le bas pendant la miction (UT-down). Nous avons explorer l'économie et la fonction de l'UT-up dans le *L. catta* une espèce où les femelles sont dominants.

Résultats et Discussion

Nous avons recueilli 240 h d'observations sur un groupe de *L. catta* dans la forêt de Berenty (au sud de Madagascar) dehors du période d'accouplement avec un échantillonnage de type « all occurrences ». Nous avons collecté différents types de comportement (marquage, déplacement, alimentation, repos, conflits) et nous avons enregistrés 191 UT-ups et 79 UT-down. Via GPS, nous avons enregistré les coordonnées des points i) fréquentés par des individus d’autres groupes et ii) fréquentés par les membres du groupe suivi. Dans le dernier cas nous avons aussi enregistré les contexte comportemental et le temps passé dans chaque lieu.

Nous avons vérifié que le marquage avec l’urine effectué par *L. catta* n’est pas un artefact de la captivité. En outre, dehors de la période d’accouplement ce type de marquage joue un rôle important dans la défense du territoire, qui est principalement effectuée par les femelles de *L. catta*. Les UT-ups était effectués et investigués plus fréquentèrent par les femelles et les UT-ups des femelles était les plus investigués en général.

Enfin, l'utilisation du signal est parcimonieux parce que l’urine était placée où et quand la probabilité de détection par les compétiteurs est plus élevé. Les UT-ups ont été effectuées dans les lieux les plus fréquentés par les individus des autres groupes et en présence de ces individus. En conclusion, nous suggérons que le marquage avec l’urine est un signal économique avec une fonction principalement territoriale.

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Multimodal Signaling in Wild *Lemur catta*: Economic Design and Territorial Function of Urine Marking

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KEY WORDS complex signals; prosimian; ringtailed lemurs; intergroup communication

ABSTRACT Urine marking has been neglected in prosimian primates. Captive studies showed that the Malagasy prosimian *Lemur catta* scent marks with urine, as well as via specialized depositions. *L. catta* urine mark, a multimodal signal, differs from simple urination in terms of different design features, including tail configuration: the tail is held up during marking (UT-up) and down during urination (UT-down). We explore economy and function of UT-up in the female dominant *L. catta*. We collected 240 h of observations on one group at Berenty (south Madagascar) during the nonmating period via all occurrences sampling. We gathered behavioral bouts/contexts (marking, traveling, feeding, resting, and fights) and recorded 191 UT-ups and 79 UT-downs. Via Global Positioning System we established the location of the places frequented i) by extragroup individuals and ii) by group members, in this case recording also behavioral context and time spent in each place. We found that *L. catta* UT-up is not an artifact of captivity. Moreover, UT-up in the nonmating period plays a role in territorial defense, which is mostly performed by females in *L. catta* society. Female UT-ups were the most investigated and UT-ups were performed/investigated more by females. Finally, signal use is parsimonious, in that urine is economically placed where and when detection probability by competitors is higher. UT-ups were performed in places most frequented by extragroup individuals and in presence of extragroup competitors (nonrandom topography and timing). In conclusion, we suggest that UT-up is an economical signal with a primarily territorial function. Am J Phys Anthropol 139:182–192, 2009. ©2008 Wiley-Liss, Inc.

Communication, an essential prerequisite for sociality, involves the transmission of signals (Wilson, 1975; Endler, 1993; Hebets and Papaj, 2005). A signal is any action or trait produced by one animal, the sender, that provides information used by another animal, the receiver. Signal transmission is often beneficial to both parties. However, in order to be effective and useful, a signal must be detected (Markl, 1983; Bradbury and Vehrencamp, 1998). Signal detectability, or how easily a signal can be distinguished from its background (Guildford and Dawkins, 1991), can be increased 1) by reducing the reaction time, which is the amount of time needed by an observer to respond to the onset of a given stimulus and/or 2) by increasing the detection probability, which is the chance of a signal to be "discovered" (Rowe, 1999; Gosling and Roberts, 2001a).

MULTIMODAL SIGNALS

Two (or more) stimuli may be more effective than one in eliciting a response from recipients. Both reaction time and detection probability can be reduced by using complex signals, which are signals made up of multiple components and often based on different sensory modalities (olfactory, acoustic, and/or visual cues; Hebets and Papaj, 2005). The reaction time can be reduced when different modalities are combined since they produce an intersensory facilitation, which, in turn, increases detection speed (Gielen et al., 1983). The reaction time of a single cue, unimodal signal, can also be shortened when an additional neutral stimulus is concurrently presented (bimodal signal; Bradbury and Vehrencamp, 1998). Detection probability can be reduced by using alerting stimulus (e.g., vigorous movements) eliciting the receiver's "selective attention" (Bradbury and Vehrencamp, 1998; Kappeler, 1998; Wyatt, 2003).

Since complex signals may provide more reliable information to receivers than simple ones, many animals produce and respond to signals made up of multiple components (Rowe, 1999). The use of multimodal signals is widespread among primates, spanning from prosimians to apes and humans (cf. Palagi et al., 2005; Lewis and van Schaik, 2007; Pollick and de Waal, 2007). Chimpanzees and bonobos use a combination of gestures and vocal/facial signals to increase social contacts (Pollick and de Waal, 2007). Gelada baboons use "vocalized yawning" for intragroup and intergroup communication (Palagi et al., unpublished data). Howling monkeys use audiovisual signaling to increase breeding opportunities (Jones and Van Cantfort, 2007). Malagasy prosimians use multimodal signals in both reproductive and nonreproductive contexts (Palagi et al., 2005; Lewis and van Schaik, 2007; Drea and Scordato, 2008). Two different studies showed that both *Propithecus edwardsi* and

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Microcebus murinus females use multimodal estrus advertisement by associating a particular vaginal morphology with vocalizations (Buesching et al., 1998; Pochron and Wright, 2003). Palagi et al. (2005) and Palagi and Dapporto (2006) described urine marking in *Lemur catta* as a multimodal signal composed of an olfactory cue (urine) and a visual cue (tail up, increasing the detection probability). Lewis and van Schaik (2007) underlined the importance of multimodal signaling in *Propithecus verreauxi*. In this species, males were divided into two categories: clean and stained chests. Stained-chested males scent marked significantly more often than clean-chested males, thus suggesting that stained chests may represent both visual and olfactory signals of dominance rank in this species.

CHEMICAL COMMUNICATION

Multimodal communication can be particularly effective when chemical signals are involved, since they impose particular constraints (Bradbury and Vehrencamp, 1998). Signalers are often not present to reinforce their depositions and they cannot know whether their scent will be detected or who the receiver will be. Moreover, involatile constituents, although prolonging the persistent time of a mark (and, possibly, increasing the detection probability), operate only over short-distances (e.g., for thick-tailed galagos see Katsir and Crewe, 1980). Despite these limitations, scents have the capacity to code for sex, age, individuality, and even populations (Katsir and Crewe, 1980; Salamon and Davies, 1998; Buesching et al., 2002) and many aspects of mammalian sociality, such as spacing, reproductive activity, competition, and predation are mediated by chemical signals (Albone, 1984; Brown and Macdonald, 1985; Wyatt, 2003).

Primate scent marking can have a variety of functions: advertisement and territorial defense (Lewis, 2005 for *Propithecus verreauxi*; Pochron et al., 2005 for *Propithecus edwardsi*; Mertl-Millhollen, 2006 for *L. catta*), intergroup communication (Lazaro-Perea et al., 1999 for *Callithrix jacchus*), advertisement of social and reproductive dominance (Heymann, 2006 for New World primates), signaling of reproductive condition (Converse et al., 1995 for *Cebuella pygmaea*; Aujard, 1997 for *Microcebus murinus*; Miller et al., 2008 for *Cebus apella*), fecundity and sex ratio regulation (Perret, 1996 for *Microcebus murinus*), and mate selection (Fisher et al., 2003 for *Nycticebus pygmaeus*).

Scent deposition can be performed via both specialized and nonspecialized secretions/excretions (Wyatt, 2003). One of the benefits of nonspecialized depositions is the reduction of the physiological cost of secretions because they use energy-free substances, such as feces and urine, more than "dedicated" glands (e.g., for carnivores see Macdonald, 1985; for callitrichines see Lazaro-Perea et al., 1999). Thus, it is not surprising that urine marking is the most ancient form of scent-marking behavior, which is found within many mammalian taxa (Albone, 1984; Epple, 1986). However, the use of urine in prosimians has been either neglected (Schilling, 1979) or considered as part of a more specialized marking system (cf. Chandler, 1975; Mertl-Millhollen, 1986; Nievergelt et al., 1998; Lewis, 2005). The use of urine for marking in prosimians has been demonstrated only recently, in captive ringtailed lemurs (*L. catta*; Palagi et al., 2005).

LEMUR CATTA: BACKGROUND AND USE OF URINE

The diurnal *L. catta* (ringtail lemur) lives in multimale–multifemale groups, and shows the most complex social organization among prosimians (Jolly, 1966). Ringtail lemurs also exhibit the most highly developed olfactory system among primates (Schilling, 1979; Epple, 1986; Kappeler, 1998; Scordato et al., 2007). Thus, *L. catta* represents an especially appropriate species to investigate the functions of urine marking as a part of broader context of chemical signaling. In *L. catta*, both sexes have apocrine and sebaceous gland fields in their genital regions and adopt distinctive handstand postures to deposit glandular secretions on substrates [genital marking (GM)]. Males possess two additional glands: paired brachial organs are pockets of sebaceous glands on the axillary surface of each shoulder that secrete a brown paste and paired antebrachial organs are apocrine gland fields located on the wrists, adjacent to a keratinized spur, that produce small quantities of clear fluid. Males sometimes mix the secretions of the latter two glands in a distinct "shoulder-rubbing" behavior (wrist-to-pit) and then deposit this mixture via "wrist marking," an audible action during which males draw their antebrachial organ and spur across a substrate (Schilling, 1974; Palagi et al., 2002; Mertl-Millhollen, 2006; Scordato et al., 2007).

L. catta are able to produce multimodal signals by combining visual and olfactory cues (Mertl, 1976; Palagi et al., 2005; Scordato and Drea, 2007; Drea and Scordato, 2008). Males, for example, impregnate their tail fur with antebrachial and brachial secretions and then waft their tail at opponents during characteristic "stink fights" (Jolly, 1966).

Palagi (unpublished data) observed that captive ringtailed lemurs deposit urine in combination with two different tail configurations, UT-up (urine is deposited with the tail held up in an evident display) and UT-down (urine is discharged with the tail only slightly raised) (Fig. 1a). Palagi et al. (2005) showed that UT-up is a form of scent marking, while UT-down, or simple urination, is not. The authors showed that, compared to UT-down, UT-up has different design features: lower quantity, seasonality, and GM possibly following urine deposition (Fig. 1b). Via both observational and experimental approaches, the same authors demonstrated that only UT-up has a marking function and is preferentially induced by the presence of foreign individuals. Moreover, lemurs showed more interest in investigating UT-up than UT-down both in the presence (behavioral data) and in the absence of visual cues (scent tests), suggesting that urine deposited by UT-up and UT-down differs not only in tail configuration, but also in chemical properties. Differences may be related to urine concentration or to a partial mix of urine with genital secretions (favored by UT-up posture), but chemical analyses are needed to confirm this hypothesis (Palagi et al., 2005). Whether urine is mixed with specialized secretions or not, the advantages of using urine hold true, since they are related to the lower cost of exploiting a nonspecialized substance.

There are limitations to captive research, as the absence of neighboring groups, interspecific competition, and predation, can affect investigation, detection, and deposition patterns of the olfactory behavior. In free living populations, individuals can largely differ in their

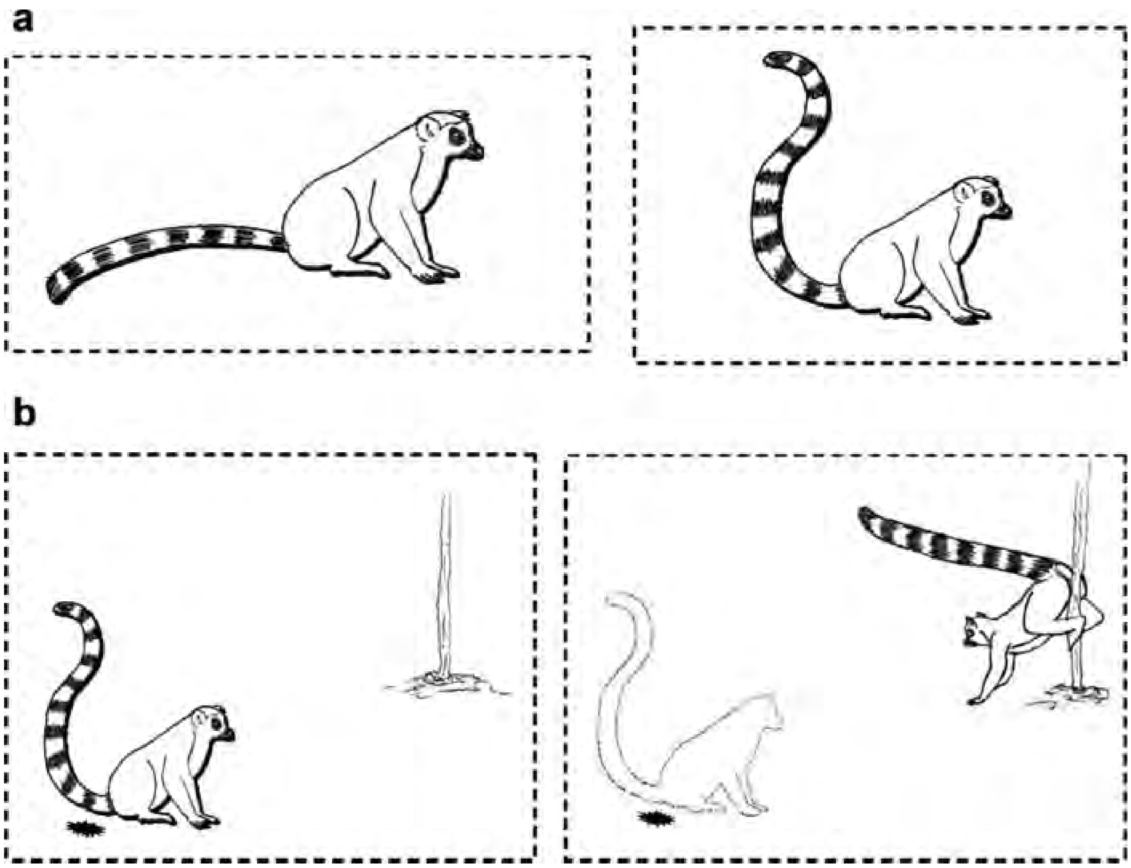

Fig. 1. Upper (**a**): Urine deposition according to the two different tail configurations: UT-down (left) and UT-up (right). Lower (**b**): UT-up alone (left) and UT-up associated with genital mark (right). The genital mark is usually performed right after UT-up on the closest sapling.

responses to chemosignals due to environmental variability, which is particularly high in Madagascar (Wright, 1999). Consequently, we extended urine-marking investigation to the wild, following a group of *L. catta* in the Berenty reserve (South Madagascar).

AIM AND HYPOTHESES

The aim of this article is twofold: a) verifying that urine marking (UT-up), with the design features described by Palagi and Dapporto (2006), is not an artifact of captivity and b) testing two alternative hypotheses on the function of urine marking.

The function of scent deposition can reflect on behavioral sex differences and on the economy of the signal. In terms of sex differences, males are generally more involved than females in defense strategies (Ralls, 1971; Pochron et al., 2005). Such strategies can be associated with direct or indirect female protection and mate guarding (Emlen and Wrege, 1986; Mesnik, 1997; Palombit, 1999; Lewis, 2005), including vigilance against predators (Wittenberger and Tilson, 1980; van Schaik and Dunbar, 1990) and defense of the feeding territory from competitors (Fashing, 2001; Thalmann, 2001; Norscia and Borgognini-Tarli, 2008). However, when females are dominant they are also particularly active in territorial and resource defense (e.g., Henschel and Skinner, 1991; Jolly and Pride, 1999; Boydston et al., 2001; Nunn and Deaner, 2004; Lewis, 2005). The term economy is defined as the "thrifty and efficient use of material/nonmaterial resources" (Merriam-Webster's Collegiate Dictionary, 10th Edition). In terms of signal economy, the energy and time costs of producing, establishing, and replenishing marks introduce constraints to the range of spatial patterns in territory scent marking (Begg et al., 2003). Consequently, the animals need to be parsimonious and to make economic "decisions" about where (and when) to place their limited number of scent marks (Gosling and Roberts, 2001b). The optimal strategy of signal deposition, in economical terms, can vary depending on signal function (Roberts and Gosling, 2001).

Urine marking can function 1) in intragroup communication for intrasexual competition and mate attraction or/and 2) in intergroup communication as a form of indirect competition over territory and resources (Albone, 1984). Considering *L. catta* biology, we present two hypotheses and for each one we consider both sex differences and signal economy.

H1—UT-up plays a major role in intergroup competition

Sex differences. Since females are dominant and highly active in territorial defense (Sussman and Richard, 1974; Budnitz and Dainis, 1975; Jolly and Pride, 1999; Nunn and Deaner, 2004), we expect that they perform and investigate (sniffing and licking) UT-ups more than males. More specifically, we expect that female UT-ups are more investigated than male UT-ups, especially by extragroup individuals.

Signal economy. Depending on the ecological correlates, signalers deposit scents according to a "geographical" strategy involving either perimeter delineation and demarcation of either food resources or of places most frequented by extragroup competitors (Kruuk, 1972; Peters and Mech, 1975; Gosling, 1987; Ono et al., 1988; Roper et al., 1993; Sun et al., 1994). In an intergroup communication perspective, we expect UT-ups to be mainly placed according to one of these strategies.

H2—UT-up plays a major role in intrasexual competition

Sex differences. Since males are the most active in olfactory communication (both in terms of investigation and deposition) (Kappeler, 1998; Palagi et al., 2003, 2004), we expect that they perform and investigate (sniffing and licking) UT-ups more than females. More specifically, we also expect that UT-ups are more investigated by males.

Signal economy. Since males counter-mark the UT-ups of females (potential mates) and males (potential competitors) regardless of group membership (Kappeler, 1998; Palagi et al., 2003, 2004; Drea and Scordato, 2008), we expect that UT-up distribution does not necessarily follow a specific territorial deposition pattern (apparently random topography).

METHODS

Study site and subjects

We conducted this study in the gallery forest of Berenty, a reserve on the Mandrare River in Southern Madagascar (for an extensive description of the forest see Jolly et al., 2006). This research was conducted in the northern part of the forest called Ankoba (S 24.99°; E 46.29°), a 40-ha secondary forest 50- to 60-years-old, with canopy at 10–15 m (except for few emergent acacias to more than 20 m) and abundance of exotic plant species. Usually, the site is characterized by two main climatic periods: a wet season from October to March and a dry season from April to September (Fig. 2, Jolly et al., 2006). However, in 2006 a drought delayed the beginning of the wet season to late November. Maximum temperature range during the study period was 36–42°C.

We collected data on eight adult individuals belonging to a single group composed of four adult males, four adult females, and one infant male; such group composition is not atypical for *L. catta* (cf. Jolly, 1972; Koyama et al., 2001). Kin relationships among group members were unknown. The individuals were well habituated to the presence of humans. Individual identification was based on sex identification and on distinctive external features (Jolly, 1966). The study group shared its territory with three males forming a temporary male-only group (or "drone's club" according to Jolly, 1966) and at least four other groups: one with more than 25 individuals mostly using the north-western part of the territory, one of 9 individuals and one of 13 individuals using the northern and the north-eastern part of the territory, respectively and one composed at least by 15 individuals exploiting the southern part of the area. Probably due to the high density of ringtailed population in Ankoba (Jolly et al., 2006), no part of the territory was exclusively used by the study group. This fact exacerbated territorial fights during intergroup encounters.

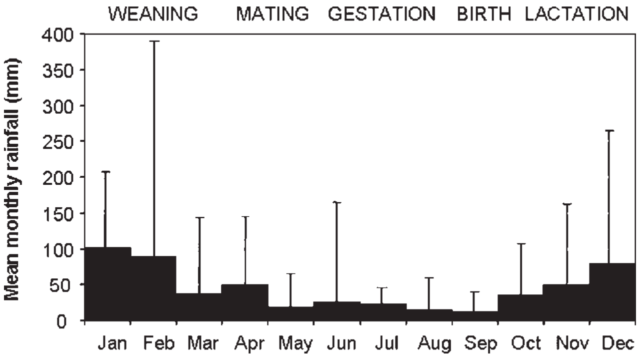

Fig. 2. Mean monthly rainfall at Berenty (period 1983–1985/1987–2004), with *Lemur catta* breeding seasonality. T-bars show maximum (Jolly et al., 2006).

Observational data

During the lactation period from mid-November to mid-December 2006, we collected 240 h of observations (30 h of observations per individual). Data collection was preceded by a training period that lasted until the observations by the four different observers (two of them were the authors) matched in 95% of cases (Martin and Bateson, 1986).

We collected data via the all occurrences sampling method (Altmann, 1974) on feeding, resting, traveling, olfactory investigation, UT-down, UT-up, and specialized marking behaviors (particularly GM). For non self-directed behaviors, we recorded both the actor and the receiver of the behavior. For UT-up, and UT-down, we recorded the behavioral context: traveling, feeding, resting, and intergroup agonistic encounters. We also recorded the presence or the absence of neighboring groups within 20 m. This distance is a conservative measure used due to the high density of *L. catta* in the study area where groups are habituated to have extragroup individuals in sight. In addition, we gathered data on deposition height and amount of urine released. We recorded whether urine was deposited in small quantity or passed in large pools (few drops vs. streams), following the procedure applied for honey badgers and pygmy lorises (Begg et al., 2003; Fisher et al., 2003). Since confidently distinguishing urine deposition in the canopy over 10–15 m can be difficult, we considered a threshold of 1 m to separate high from low depositions, thus avoiding possible observational bias. In particular, if urine was deposited at less than 1 m from the ground, such deposition was labeled as "low"; if urine was released from 1 to 5 m, such deposition was labeled as "high." Depositions performed over 5 m, although collected, were excluded from the analyses.

UT-up can be either associated or not with a genital mark (Palagi et al., 2005; Palagi and Dapporto, 2006): the genital mark, when present, is usually deposited right after UT-up on the closest sapling (Fig. 1b); the distance can range from a few centimeters to a few meters depending on vegetation distribution. To evaluate the temporal association of GM with urine deposition, we scored the frequency with which each animal performed GM within 10 s after UT-up (UT-up + GM) and UT-down (UT-down + GM). The measure of 10 s was applied following Palagi et al. (2005). Then we divided the number of bouts of UT-up + GM and UT-down + GM by the total number of UT-up and UT-down performed, respectively.

We scored olfactory investigations of UT-up and UT-down in order to examine the monitoring of the two kinds of deposition. Olfactory investigation included both sniffing and licking (Palagi et al., 2002). One of the observers monitored the place for 10 min after deposition, following the measure suggested by Kappeler (1998), and recorded if the urine released was investigated by other group members (in this case, registering the identity of the animal) or extragroup individuals (in this case, registering the sex of the animal).

Use of space and olfactory signal distribution

We established the location of trees/places frequented by the study subjects for resting, feeding, and urine deposition (UT-up and UT-down) by using a Global Positioning System (GPS, Garmin eTrex). All places were marked via colored ribbons.

During the whole study period we collected GPS positions for each resting and feeding tree (97 positions in total), for each UT-down (30 positions in total) and UT-up (58 positions in total). Several UT-ups and UT-downs were performed on the same places in different days. Data points were subsequently plotted as UTM coordinates (decimal degrees). We processed the data using a geographical information system (ARCVIEW GIS, 3.2a) in combination with the Animal Movement Analysis Arcview Extension (Wronski and Apio, 2005). According to data points (feeding, resting, and urine deposition), the study group frequented a home range of 2.26–2.62 ha (95% Kernel, respectively; 100% minimum convex polygon; Southwood, 1996; Seaman et al., 1998). We also measured the time (hours) spent by the animals in each place marked with the ribbons and for which the GPS position was recorded. Moreover, we marked and recorded the GPS positions of the places where we saw intergroup encounters. Finally, while two observers were following the study group for daily observations, the others patrolled, every 2 h, the area covered by the group the day before (daily range). The two "patrol observers" recorded GPS positions of other groups present in the range of the study group and recorded the number of sightings for each marked place (absolute frequency). This measure was used to evaluate extragroup frequentation of the study group home range. All places were visited by the "patrol observers" a fixed number of times ($n = 12$).

Statistics

Nonparametric statistics (Siegel and Castellan, 1988) were applied both to the analyses performed at the individual level (due to the small sample size, $N = 8$) and to the correlations involving the spatial distribution of urine depositions (due to sample deviation from normality; Kolmogorov–Smirnov test: location points considered for correlations $N = 154$; $Z = 4.34$–5.74, $P = 0.001$; UT-up locations, $Z = 3.14$, $N = 58$, $P = 0.001$). The significance level of probability was fixed at 0.05 and adjusted downward with Bonferroni correction when the same variable was involved in repeated correlations. Exact values of probability were used according to Mundry and Fischer (1998). In particular, the Wilcoxon signed rank test, performed within adult individuals, was used to compare the differences between UT-up and UT-down (deposition and reception frequencies, height, GM association, urine quantity, time spent on the spot after deposition, behavioral contexts, and presence of extragroup individuals). Additionally, the Mann–Whitney *U* test was applied to check for possible differences in urine deposition and reception between males and females within the study group. Moreover, the Spearman Rank Order Correlation was employed to verify the possible correlation between UT-up/UT-down frequency and either the time spent or the extragroup presence on each location. Finally, the Spearman test was used to evaluate the correlation between the frequency of UT-up investigation by extragroup individuals and the frequency of extragroup presence in each location.

RESULTS

We collected a total of 191 UT-ups (mean 23.89 ± 10.20 SD), 79 UT-downs (mean 9.88 ± 2.17 SD), and 396 GMs (mean 49.50 ± 29.75 SD). UT-downs increased by 150–200% in the days following the first seasonal rain (delayed to 30 November in 2006). Still, UT-up was performed much more frequently than UT-down (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 0, $P = 0.008$). UT-up differed from UT-down according to various features. During UT-down, lemurs discharged streams of urine more frequently than drops (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 0, $P = 0.008$); whereas in UT-up drops were released more frequently than streams of urine (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 0, $P = 0.008$). UT-up was preferentially performed less than 1 m from the ground (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 0, $P = 0.008$), whereas UT-down was performed preferentially on branches >1 m from the ground (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 1, $P = 0.02$). UT-up was significantly more investigated than UT-down (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 0, $P = 0.008$): in particular UT-up was investigated at least once in 83.21% of cases within 10 min after deposition, whereas UT-down was never investigated. 95% of UT-up was investigated within the first minute after deposition. Moreover, UT-up not followed by GM and UT-up + GM were performed and investigated with comparable frequencies (Exact Wilcoxon Signed-Ranks test; deposition: $N = 8$, $T = 8$, ties = 1, $P = 0.38$; investigation: $N = 8$, $T = 9.5$, ties = 1, $P = 0.50$). Percentages of performance and investigation of UT-up and UT-down are reported in Table 1.

Sex differences

We found no difference in the frequency of UT-down between males and females (Exact Mann–Whitney *U* test, $n_M = 4$, $n_F = 4$, $U = 2$, $P = 0.11$). Females performed 55.76% of UT-downs and males 44.24%. However, we found that intragroup females performed more UT-ups than males: 65.41% (females) versus 34.59% (males). Females also performed UT-up more frequently than intragroup males; Exact Mann–Whitney *U* test, $n_M = 4$, $n_F = 4$, $U = 0$, $P = 0.03$). Intragroup females were also more active in investigating UT-up than males (Exact Mann–Whitney *U* test, $n_M = 4$, $n_F = 4$, $U = 0$, $P = 0.03$).

Moreover, female UT-up was generally more investigated than male UT-up (Exact Mann–Whitney *U* test, $n_M = 4$, $n_F = 4$, $U = 0$, $P = 0.03$): this result was due to

TABLE 1. Total percentages UT-up and UT-down performed and investigated a) in presence extragroup versus intragroup individuals, b) by resident females versus males, and c) in association with GM

		Performance (%)	
	By females/males	With extragroup individuals	Followed by GM
UT-up	65.4/36.4	72.7	58.2
UT-down	55.8/44.2	36.7	1.8
		Investigation (%)	
	By females/males	By extragroup individuals	Not followed by GM
UT-up	43.4	30.8	41.3
UT-down	0/0	0	0

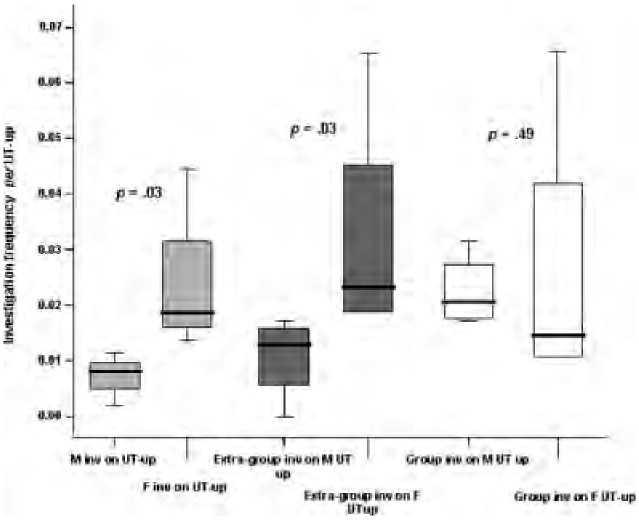

Fig. 3. Frequency of 1) male and female investigation on UT-ups (light gray), 2) extragroup individuals' investigation on male and female UT-ups (dark gray), and 3) group members' investigation on male and female UT-ups (white).

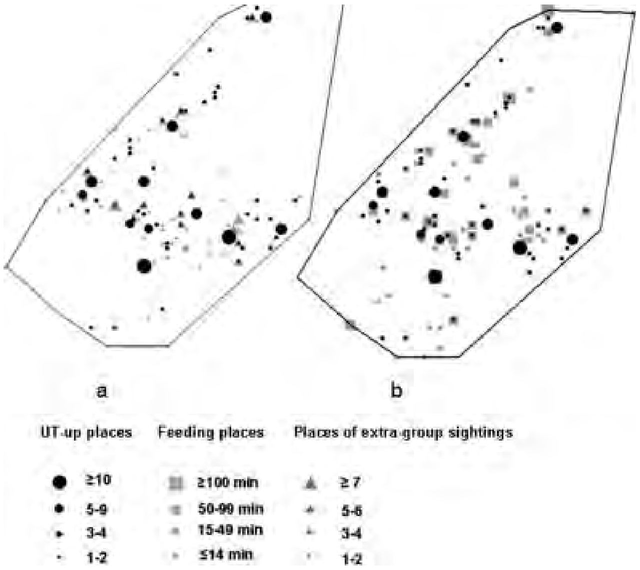

Fig. 4. Group home range with GPS points mapped on it. The map shows the spatial distribution of UT-up depositions combined to the places where extragroup individuals were observed (**a**) and where group members fed (**b**). The ranges of UT-up frequency (black circles), time spent feeding (gray squares), and absolute frequency of extragroup sightings (gray triangles) are also indicated. Increasing frequency and time are indicated by the different size of symbols (see figure legend).

extragroup individuals. Group members did not show any preference in investigating female or male UT-ups (Exact Mann–Whitney U test, $n_M = 4$, $n_F = 4$, $U = 5$, $P = 0.49$), whereas extragroup members preferentially investigated female UT-ups (Exact Mann–Whitney U test, $n_M = 4$, $n_F = 4$, $U = 0$, $P = 0.03$) (see Fig. 3). Additionally, UT-up depositions were more frequently sniffed by extragroup females than males (Exact Wilcoxon Signed-Ranks test, $N = 8$, $T = 0$, ties = 2, $P = 0.031$). Finally, immediately after intergroup fights we never observed UT-down whereas we always observed at least one UT-up deposition (min: 1; max: 3; 72.67% of UT-ups were performed in presence of other groups). UT-up depositions were exclusively performed by females and have been shown by all females when at least one of the extragroup individuals was in sight.

Signal timing and distribution

The frequency of UT-ups performed on the locations mapped via GPS correlated with the number of sightings (absolute frequency) of extragroup individuals in such locations (Spearman correlation with Bonferroni correction ($\alpha/2$): $r_s = 0.394$, $N = 154$, $P < 0.001$), but did not correlate with the time spent by intragroup members at the same locations for feeding and resting (Spearman correlation with Bonferroni correction ($\alpha/2$): $r_s = 0.08$, $N = 154$, $P = 0.31$). Such results are visualized in Figure 4, which shows that UT-up locations match better with the places frequented by extragroup individuals (4a) than with the feeding places (4b). Matching also involves the frequency of behaviors as well.

The locations where UT-ups were performed more frequently matched with the places most frequented by extragroup individuals (in Fig. 4, increasing frequency is indicated by increasing size of symbols). On the other hand, the locations where UT-up were performed more frequently did not match with the places where the animals spent more time feeding (4b; increasing feeding time is indicated by increasing size of symbols).

UT-down followed the opposite trend being preferentially placed in the areas where the study group stayed longer (Spearman correlation with Bonferroni correction ($\alpha/2$): $r_s = 0.276$, $N = 154$, $P < 0.001$), but not in the locations where extragroup individuals were seen more often (Spearman correlation with Bonferroni correction ($\alpha/2$): $r_s = 0.24$, $N = 154$, $P = 0.003$).

At the points where UT-up was placed, a significant correlation was found between the frequency of UT-up investigated by extragroup receivers and extragroup frequentation (Spearman correlation: $r_s = 0.32$, $N = 58$, $P = 0.02$).

On a temporal scale, UT-up was preferentially performed when extragroup individuals were in view (less

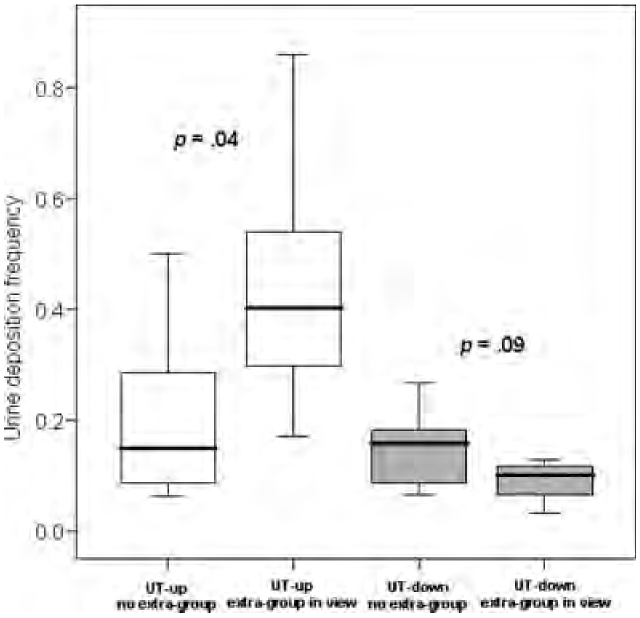

Fig. 5. Frequency (per hour) of UT-up (white boxes) and UT-down (gray boxes) deposition in the presence and absence of extragroup individuals.

than 20 m away; Exact Wilcoxon Signed-Ranks test, N = 8, T = 3, ties = 0, P = 0.039), whereas the UT-down was performed regardless of the presence of extragroup individuals (Exact Wilcoxon Signed-Ranks test, N = 8, T = 3, ties = 2, P = 0.09) (see Fig. 5).

UT-up and UT-down frequencies differed during traveling (UTup > UTdown; Exact Wilcoxon Signed-Ranks test, N = 8, T = 0, ties = 1, P = 0.02), but not during resting and feeding (Exact Wilcoxon Signed-Ranks test, N = 8, T = 1, ties = 2, P = 0.06). UT-up was performed more frequently during traveling (in open spaces along the main pathways) than during resting and feeding contexts (in the canopy; Exact Wilcoxon Signed-Ranks test, N = 8, T = 0, ties = 0, P = 0.008), whereas the frequency of UT-down did not differ in the two contests (Exact Wilcoxon Signed-Ranks test, N = 8, T = 17, ties = 0, P = 0.95).

DISCUSSION

Urine marking in the wild

Chemical communication with signals from specialized glands in *L. catta* has received attention spanning 40 years of lemur research, which led to a profound knowledge of significance, modalities, and levels of recognition (species, group, sex, and individual) (Jolly, 1966; Dugmore et al., 1984; Mertl-Millhollen, 1988; Kappeler, 1990, 1998; Ramsay and Giller, 1996; Sauther et al., 1999; Gould and Overdorff, 2002; Hayes et al., 2004; Palagi et al., 2004; Scordato and Drea, 2007). Possibly hidden by the complex system of specialized glandular secretions of *L. catta*, the use of urine had been disregarded until the work of Palagi et al. (2005).

Our results confirm and expand previous findings by Palagi et al. (2005) and Palagi and Dapporto (2006) on the UT-up. In the wild, we found that UT-up maintains the same deposition features found in captivity (such as urine deposited in drops and tail-up position). GM is the most common form of specialized scent marking: not only is it the only one performed by both sexes, but it is also often used in combination with other depositions (e.g., wrist mark, Palagi, personal observation).

Palagi et al. (2005) described a strict temporal and spatial association between UT-up and GM. Theoretically the signal of GM could prevail over UT-up, because GM is a specialized deposition. In this perspective, it was necessary to show that UT-up does not represent an incidental deposition but a signal *per se*. Here, we found that UT-up associated with GM (Fig. 1b) and UT-up alone were performed with comparable frequencies. Moreover, UT-up not followed by GM was investigated as much as UT-up followed by GM. Such results clarify, for the first time that, the association with GM does not lead to an increase of UT-up effectiveness. Thus, UT-up and GM have to be considered as separate signals and should not be grouped together. Since the aim of this study is to analyze economy and function of urine marking, from now on we will specifically focus on UT-up depositions. GM, associated or not with other depositions, is the subject of a companion study comparing different kinds of scent marking in *L. catta*.

The tail display by *L. catta* during UT-up may increase the probability of investigation by conspecifics by indicating the deposition place. Investigation was higher for UT-up than for simple urination (UT-down), which lacks the visual stimulus. The importance of the tail up as an alerting stimulus in enhancing signal detection becomes clear with the finding that 95% of UT-ups were investigated within the first minute. In the same species, Kappeler (1998) found that 62% of the specialized scent marks, often combined with visual cues, were investigated with a median latency of 30 s. Thus, assessing the odor quickly is important, before it vanishes.

Sex differences

In terms of sex differences, we found that UT-up follows a pattern different from that described for other kinds of scent marking in *L. catta*. Because of the lack of ante-brachial and brachial glands in females, male antebrachial marking and tail anointing and waving are multimodal signals restricted to males (Jolly, 1966; Mertl, 1976; Kappeler, 1998). GM, although present in both sexes, is performed more by adult females than adult males (Kappeler, 1990, 1998), but the latter are more active in investigating genital marks (Gould and Overdorff, 2002; Palagi and Dapporto, 2007). Moreover, males scent mark at higher overall rates when all forms of specialized scent marking are pooled (Kappeler, 1990). Additionally, males investigate conspecific specialized depositions at much higher rates than do females (Scordato and Drea, 2007). Based on our results, UT-up is the only form of scent marking in which both deposition and investigation rates are higher in females than in males. In an evolutionary perspective, the reduced use of UT-up by males may be due to the fact that they have partly "replaced" this basal form of scent-marking (plesiomorphism) with specialized brachial and antebrachial secretions (apomophism), which are missing in females.

The fact that females were more active in performing and investigating UT-up (cf. Fig. 3, Table 2) suggests a territorial function of this multimodal signal, because females are the real "defending sex" in *L. catta* (Jolly, 1966). It is interesting to notice that when females are the philopatric sex and dominate males in aggressive encounters, they also show higher levels of olfactory

TABLE 2. List of predictions and outcomes

Hypotheses	Predictions: Sex differences	Predictions: Signal economy	Outcome
H1: UT-up mainly used for intergroup competition	Females perform and investigate UT-ups more than males. Female UT-ups are more investigated than male UT-ups	Non random topography of signal deposition	Confirmed
H2: UT-up mainly used in intrasexual competion	Males perform and investigate UT-ups more than females. UT-ups are more investigated by males.	Signal deposition apparently random	Not confirmed

TABLE 3. Main zones within the home range of the study group

Zone	Vegetation	Area	Location	Main food species and abundance
Part A	Sisal plantation	0.25 ha	North-west	5% *Salvadora angustifolia*
Part B	Plantation of Didieraceae	0.9 ha	West	95% *Allouadia procera*
Part C	Open space and road	0.2 ha	Center	97% *Cordia sinensis*
Part D	Secondary forest	1.3 ha	East, south-east, north-east	20% *Rinorea greveana* 18% *Celtis* spp 11% *Pithecellobium dulcae* 6% *Tamarindus indica*

For each part we reported: vegetation type, area included in the home range, location in the home range, main food species, and their abundance. Part A is constituted almost exclusively by sisal, with the exception of *Salvadora angustifolia. Allouadia procera* and *Cordia sinensis* are present only in part B and C, respectively (Norscia and Palagi, unpubl. data).

activity linked to territorial defense (Jolly, 1966; East et al., 1993; Ostner et al., 2003). For example, hyena females, which initiate and lead most of the territorial activities, are more involved in territorial scent marking than males (Henschel and Skinner, 1991; Boydston et al., 2001). In the Kirindy forest (west Madagascar), Lewis (2005) found that Verreaux's sifaka males mainly scent marked to communicate their presence to other groups (clean-chested males) or for mate-guarding (stained-chested males), whereas females scent-marked in areas where their home range overlapped with the home range of other groups, possibly claiming "ownership." Moreover, in the Edward's sifaka, Pochron et al. (2005) found that female scent marks were mostly involved in intrasexual competition and territoriality (Pochron et al., 2005). Finally, Mertl-Millhollen (2006) underlined the importance of female scent marking for resource and territorial defense by *L. catta* females. She predicted that female scent marking, being a key component of intergroup fights, would occur at a higher rate during confrontations. This prediction was confirmed for female GM, in agreement with previous literature (Jolly, 1972; Sussman and Richard, 1974).

We also found that extragroup individuals selectively investigated female UT-up (see Fig. 3) with extragroup females particularly active (Table 2). This evidence further supports the role of urine in territorial defense. Although a group is rather permeable to extragroup males (visiting and migrating males: Jolly, 1966; Sauther, 1991), resident females make the group impassable for alien females, in order to protect resources (Nakamichi and Koyama, 1997). Males probably consider alien females not as competitors but as possible sexual partners (Palagi and Dapporto, 2007). Instead, a female is interested in the reproductive status of another female mostly in the mating period and almost exclusively within the same group (the dominant female can manipulate the estrus of subordinate females by limiting their access to information on her own estrus) (Palagi et al., 2003, 2004).

Signal economy

Because of time and energy constraints, animals need to maximize the probability of mark detection, which in turn depends largely on the interaction between movements of intended receivers and the spatial deployment of scent marks (Gosling, 1986). When scent marks are used to claim territory ownership, they can be placed a) to delineate the perimeter of an area characterized by relatively homogenous resources (e.g., see Kruuk, 1972 for hyenids; Macdonald, 1985 for canids; Smith et al., 1989 for felids; Rosell et al., 1998 for rodents; Gosling, 1987 for artiodactyls); b) to demarcate food resources clustered in a specific zone (e.g., for antelopes see Ono et al., 1988; for badgers see Roper et al., 1993); and c) to demarcate the pathways and places most frequented by extragroup competitors (e.g., for wolves see Peters and Mech, 1975; for deer see Sun et al., 1994).

We found that UT-up was preferentially deposited by *L. catta* during traveling and in the places that were more frequented by extragroup individuals (see Fig. 4), thus reinforcing the hypothesis that UT-up is mainly used in intergroup competition (Table 2). This strategy is consistent with the fact that resources, in Ankoba, are heterogeneous (Table 3) and that lemur home ranges are widely overlapping due to the high density in this area (Jolly et al., 2006; Norscia and Palagi, 2008). Habitat heterogeneity can channel animal movements leading to the tendency for marks to be placed along the pathways that are most likely to be frequented by extragroup individuals (Peters and Mech, 1975; Gosling and Roberts, 2001a). Moreover, perimeter delineation is ineffective when the area of exclusive use is reduced, as in the case of our study group.

In terms of timing, 72.67% of UT-ups were performed when extragroup members were in view (within 20 m; cf. Fig. 5). By contrast, UT-down was placed *ad libitum*, regardless of the presence of extragroup individuals, and proportionally more where the animals stayed longer. This finding underlines the fundamental role of the visual component already suggested in this species by a series of elegant experiments carried out by Mertl (1976). When *L. catta* males could sniff but could not see each other, no tail marking or tail waving occurred. On the contrary, when the visual contact was permitted, the behavioral response was not only high (tail marking and waving performed at high frequency), but also complex (tail marking and waving were often combined together). *L. catta* does not always check for the presence of alien individuals when using the alerting stimulus prior to scent mark. This is probably the reason why a certain number of UT-ups were performed without extragroup members in view. Such stimulus is not dissociated from the olfactory component (urine), which stays longer and, as explained above, is performed where the detection probability is higher. Scent messages act as olfactory bulletin boards that persist in the absence of the signal sender (Scordato et al., 2007). The temporal and spatial association of UT-ups with the presence of extragroup individuals supports a major intergroup communication role for the UT-up. Unlike other lemurs like *Propithecus verreauxi*, *L. catta* groups are almost isolated in terms of reproductive and sexual competition: in contrast with *P. verreauxi*, *L. catta* females were seen mating only with males of the same group in the mating period (Brockman, 1999; Palagi et al., unpublished data). In this respect, intergroup communication in *L. catta* is more related to territory than to reproductive/sexual competition.

In short, *L. catta* economize on UT-up depositions by both maximizing the detection probability (placing depositions on the pathways most patrolled by potential receivers) and minimizing the reaction time (depositing urine in presence of extragroup individuals). Such a strategy, adopted by different mammals (Brashares and Arcese, 1999; Gosling et al., 2000), can be particularly effective in habitats characterized by high temperatures and humidity (like Berenty; Fig. 2), which strongly affect the persistence of chemosignals (Alberts, 1992; Bradbury and Vehrencamp, 1998).

The fact that the animals performed UT-up in the places most frequented by extragroup competitors does not indicate *per se* that this strategy of signal deposition is effective. To fill this gap, we also verified that the majority of the UT-ups performed were actually sniffed and/or licked at least once (83.21%) within 10 min after deposition, and that animals investigated (sniffed/licked) UT-up at higher rates in the places most frequented by other groups. Consequently, the consideration of both receivers' and senders' perspectives led us to the conclusion that such strategy is successful.

Although UT-up communication for reproductive/sexual purposes cannot be excluded, sex differences and signal economics (interpreted in the light of *L. catta* biology) converge in indicating that UT-up is mainly used for territorial purposes. The results in the wild fit with the directions provided by captive research (Palagi et al., 2005). Scent tests revealed that *L. catta* can discriminate between urine of their own group and that belonging to a foreign one (Palagi et al., 2005). In addition, Palagi et al. (2005) observed an increase of UT-up deposition in presence of a dummy. Besides, UT-up was preferentially placed in the zone of the enclosure where two groups, although separated, were in visual and olfactory contact.

Compared to UT-up, specialized secretions seem to be used more loosely, being placed not only in intergroup confrontation places, but also on food resources (Mertl-Millhollen, 1988, 2006; Kappeler, 1990, 1998). Our results are restricted to one group and to the nonmating period, so we cannot exclude that UT-up deposition pattern varies according to different contexts and periods (mating season or sites characterized by clustered resources). However, the fact that urine is more selectively used than any other forms of scent marking could be the result of a major physiological constraint: the liquid loss intrinsic in urine deposition. Such loss is much lower when condensed gland secretions are used (cf. Wyatt, 2003) and is more costly in the dry season. As a matter of fact, UT-downs doubled or tripled in the days after the first seasonal rain. The low rainfall levels and the high temperature in the study period may have further constrained the lemurs to conserve fluids, thus leading to a very thrifty use of urine both in terms of topography and timing.

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L'étranger devient familier: les primates strepsirrhiniens dans la nature suivre la xénophobie à travers le jeu.

Introduction

Le pouvoir du jeu en limitant la xénophobie est un phénomène bien connu chez l'homme. Pourtant, la preuve chez les animaux sociaux reste faible. Ici, nous visons à déterminer si le comportement de jeu favorise la tolérance sociale envers les étrangers dans les groupes les plus basale de primates, les strepsirrhiniens. Nous avons observé deux groupes de lémuriens en liberté de l'espèce *Propithecus verreauxi* (sifaka de Verreaux) pendant la saison des amours. Les données ont été recueillies aussi sur neuf males d'autres groups qui visitait occasionnellement le groupe d'étude. Nous avons comparé la distribution du jeu, toilettage (*grooming*), et les interactions agressives dans trois conditions différentes: OUT (interactions entre individus résidents et individus d'autres groups), IN (interactions entre résidents en présence d'individus d'autres groupes) et BL-IN (interactions entre résidents en absence d' individus d'autres groups).

Resultats et Discussion

Le comportement de jeu était plus fréquent entre les résidents et les mâles d'autres groupes que entre résidents et résidents. Par contre le toilettage, indicateur de bonnes relations entre individus, était plus fréquent entre les individus résidents dans chaque groupe d'étude. Le taux d'agressivité entre les mâles résidents et les visiteurs d'autres groupes était nettement supérieur à ce entre les résidents. Cependant, les agressions entre les mâles résidents et les males d'autres groupes diminuaient de façon significative après la première session de jeu, devenant comparables au niveau d'agression entre résidents.

La présence d'étrangers dans un groupe bien établi implique l'apparition de nouvelles circonstances sociales, que les mâles de sifaka abordent à travers de deux tactiques différentes: les mâles résidents s'engagent dans des sessions de toilettage avec les autres résidents et jouent avec les males 'étrangers'. Les fréquences plus élevées de toilettage ont étés enregistrées pendant la visite des individus externes et cela représente probablement un bouclier social adoptée par les mâles résidents pour rendre les relations d'affiliation préexistantes bien évident au 'public étranger'. Par contre, le jeu était la plupart du temps destiné aux mâles inconnus et, pour cette raison, ce comportement chez les sifakas adultes semble avoir un rôle plus important dans la gestion des situations sociales nouvelles que dans le maintien de relations déjà existantes. En particulier, nos résultats indiquent non seulement que le jeu est l'interface entre les résidents et les étrangers, mais aussi qu'il a une fonction spécifique dans la réduction de la xénophobie. En conclusion, le jeu semble être un mécanisme brise-glace dans le processus critique qui transforme un individu étranger dans un familier.

Stranger to Familiar: Wild Strepsirhines Manage Xenophobia by Playing

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Abstract

The power of play in limiting xenophobia is a well-known phenomenon in humans. Yet, the evidence in social animals remains meager. Here, we aim to determine whether play promotes social tolerance toward strangers in one of the most basal group of primates, the strepsirhines. We observed two groups of wild lemurs (*Propithecus verreauxi*, Verreaux's sifaka) during the mating season. Data were also collected on nine visiting, outgroup males. We compared the distribution of play, grooming, and aggressive interactions across three conditions: OUT (resident/outgroup interactions), IN (resident/resident interactions in presence of outgroups) and BL-IN (baseline of resident/resident interactions in absence of outgroups). Play frequency between males was higher in OUT than in IN and BL-IN conditions; whereas, grooming was more frequent in IN than in OUT and BL-IN conditions. Aggression rates between resident and outgroup males were significantly higher than those between residents. However, aggressions between resident and outgroup males significantly decreased after the first play session and became comparable with resident-resident aggression levels. The presence of strangers in a well-established group implies the onset of novel social circumstances, which sifaka males cope with by two different tactics: grooming with ingroup males and playing with outgroup ones. The grooming peak, concurrently with the visit of outgroups, probably represents a social shield adopted by resident males to make their pre-existing affiliation more evident to the stranger "audience". Being mostly restricted to unfamiliar males, adult play in sifaka appears to have a role in managing new social situations more than in maintaining old relationships. In particular, our results indicate not only that play is the interface between strangers but also that it has a specific function in reducing xenophobia. In conclusion, play appears to be an ice-breaker mechanism in the critical process that "upgrades" an individual from stranger to familiar.

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Introduction

> *You can discover more about a person in an hour of play than in a year of conversation*
> Plato, The Republic

Xenophobia (from Greek: *xénos*, foreign and *phobos*, fear) literally indicates aversion to strangers and in its extreme form is expressed through a cooperative violent reaction of the residents toward strangers. This phenomenon, addressed as "xenophobia principle" by socio-biologists, is widespread in animals ([1], p. 286). In primates, xenophobic reactions include agonistic chasing (in sifaka: *Propithecus verreauxi*;[2]), target aggressions (in ring-tailed lemurs: *Lemur catta*; [3]), cooperative attacks (in rhesus monkeys: *Macaca mulatta*; [4]), coalitionary killing (in chimpanzees, *Pan troglodytes*; [5]) and warfare (humans, *Homo sapiens*; [6]).

The power of play in limiting xenophobia by promoting tolerance in humans was known since ancient times. According to Greek mythology, Apollo, queried through Delphi's oracle, told Ifitos (the King of Elis) that the wars devastating Peloponnese would be ended by staging a sport competition at Olympus. After the Olympic Games were re-established (and this is where the myth ends and history begins), the longest-standing peace accord in history (the Olympic Truce) was signed between the regions of Peloponnese [7]. The role of human play in limiting xenophobic aggressions is more than anecdotal and does not apply only to structured play. This is not surprising, considering that complex social play represents one step of play ontogeny, which begins with spontaneous play fighting and reaches its climax with the production of sophisticated games [8]. In children, play fighting (or rough and tumble) leads to the direct inhibition and regulation of aggression, thus improving social integration [9]. Hunter-gatherer societies where play (both with rules and without) is used in social practices (religion, bargaining, children's education, etc.), show a more fluid, democratic structure and are more open to new incomers [10].

Animals, as well as children, do follow rules during social play but such rules are flexible and negotiated by players *hic et nunc* ("here and now") [11]. Flexibility and improvisation that characterize social play are considered to be the locomotive of cognitive and behavioral innovation [12].

In juveniles, play can have long term positive effects by improving motor and psychosocial skills [13]–[16]. Among adults, play appears to be especially fruitful at a short term level for manipulating specific social situations (tension reduction, cohesiveness increase, low risk relationship assessment) [17]–[22].

Thus, what is really important in adult-adult play is animals' ability to opportunistically use play in the most appropriate way. Consequently, play effectiveness does not lie on quantity (how much adults play) but on quality that is how (social context), with whom (play-mate choice), and when (timing) adults play.

While juvenile play is ubiquitous among primates, adult play is less frequent and is scarcely documented in quantitative terms [18], [21], [23], [24]. Adult play is unrelated to phylogenetic relationships among species (showing a patchwork distribution among primate taxa) [25], and strongly affected by social organization and inter-individual bonding quality [20]. Focusing on adults may be a first step toward a deeper understanding of the short term benefits of play [26].

Adult play benefits seem to be *maxima* in case of uncertainty in social relationships among individuals [26], such as when mating involves unfamiliar subjects [27]–[29] and/or when group composition is fluid, with some group members meeting each other occasionally (e.g. in fission-fusion societies: *Homo sapiens*, [10]; *Pan* spp., [30], [31]; *Ateles* sp., [32]; *Cacajao* sp., [33]). During ephemeral and sporadic associations, animals have to engage in behavioral interactions to establish or re-establish a sufficient level of familiarity [34].

Here, we aim to determine whether play is used to manage xenophobia in the most basal group of primates, the strepsirhines. To test this hypothesis, we selected the sifaka, *Propithecus verreauxi*, a species where adult-adult play occurs. Most strepsirhines are characterized by either dispersed sociality (solitary or pair-living individuals) [35] or social xenophobic groups completely sealed to outsiders (e.g. *Lemur catta*, [3]). Sifaka live in cohesive multi-male/multi-female groups and show temporary variations in group composition, especially during the mating season [36]–[39]. In this period, males can start roaming and visiting other groups in search of receptive females, which experience a single estrus period per year (up to 72 h). Subjects of both sexes can mate with multiple partners in their own and neighboring groups [40], [41]. Mate choice is a prerogative of females, due to their dominance over males [39], [40].

The plasticity characterizing sifaka groups provides a rare opportunity to determine if adult play facilitates the integration of unfamiliar individuals. We tested the following predictions.

Prediction 1 – *Can play be considered as a purely affinitive behavior?*

Grooming is used as the main social cement within primate social groups and it is typically, mostly exchanged between individuals sharing good relationships [42]. Different authors have shown that social play, as well as grooming, can work to maintain relationships between subjects with pre-existing social bonds [24], [30]. On the other hand social play, can also involve unfamiliar individuals, and we therefore hypothesize that play is not solely affinitive. If so, we expect grooming and play not to follow the same distribution patterns, especially when unfamiliar subjects are involved (Prediction 1).

Prediction 2 – *Play for courtship*

When a male meets an unfamiliar female immediately before it is receptive (courtship context), play appears to reduce aggression, thus establishing familiarity suitable for more relaxed and successful copulations [27]–[29].

If social play is also used by outgroup, sifaka males for courtship, we predict i) higher levels of play between resident females and outgroup males compared to resident females and males; and ii) higher motivation to engage in social play by outgroup males than resident females

Prediction 3 – *Play for promoting tolerance and limiting xenophobia*

In *Propithecus verreauxi* intergroup encounters are common at feeding sites within overlapping home-ranges [2],[43]. Moreover, resident males have been observed to sometimes form coalitions to keep extra-group males out and to prevent them from mating with resident females [44]. However, residents also exhibit behaviors that appear to facilitate group membership for strangers [45], possibly due to the potential benefits provided by extra males in groups of sifakas such as increased vigilance and resource defense [46]. Hence, under certain circumstances males need to modulate and moderate their xenophobic response, in order to mediate between acceptance and rejection of outgroup males trying to break into the group.

In this case of extreme social uncertainty, play may be used as an ice-breaking mechanism to promote tolerance and limit xenophobia. If so, we expect i) higher levels of play between ingroup and outgroup males (more "unfamiliar" to each other) than between ingroup males; ii) comparable levels of play initiation between outgroup and ingroup males; iii) a decrease of agonistic interactions after play between ingroup and outgroup members.

Prediction 4 – *Does familiarity affect play intensity?*

Animals can fine-tune play sessions, in terms of intensity, according to play mate, context (more or less risky), and timing [47]. Play can be graded along a gradient of intensity, going from gentle play, involving no body contact (e.g. play run) or a sequence of contact and/or no-contact patterns, to rough play (or rough and tumble), involving fighting with a series of body contact patterns normally performed in rapid succession (e.g. biting, pushing, pulling, rolling, falling on the ground) [26], [48]. In its roughest version, play is one of the most sophisticated forms of social interaction, during which playmates have to trust each other to maintain play rules and avoid escalation into serious fights [49]. If, in the study species, social play also implies trust between individuals rough play should be more common among ingroup males than between outgroup and ingroup males.

Results

Play and grooming distribution

We compared play and grooming levels across three conditions: male-OUTmale (interactions between males of the observed groups and outgroup males), male-INmale (interactions between males of the observed groups during the visit of outgroup males), and male-BL-INmale (control variable including the interactions between males of the observed groups recorded in absence of outgroup males).

We found a significant difference in the play distribution across the three conditions: male-OUTmale, male-INmale, male-BL-INmale (Friedman's $\chi^2 = 13.034$; df = 2; $N_{males} = 8$; $p = 0.001$). Dunnett's test revealed a significant difference between male-OUTmale *vs* male-INmale (OUTmale > INmale: $q = 2.01$; $p < 0.05$) and male-OUTmale *vs* male-BL-INmale (male-OUTmale > male-BL-INmale $q = 1.97$; $p < 0.05$); conversely, no difference was found between male-INmale *vs* male-BL-INmale ($q = 1.03$; $p > 0.05$) (Fig. 1). Outgroup and ingroup males initiated play sessions at similar rates between each other (Wilcoxon's $T = 8.50$; ties = 3; $N_{males} = 8$; $p = 0.75$). Grooming distribution significantly differed according to male-OUTmale, male-INmale, male-BL-INmale conditions (Friedman's $\chi^2 = 12.97$; df = 2; $N_{males} = 8$; $p = 0.0001$). Specifically, Dunnett's test revealed a

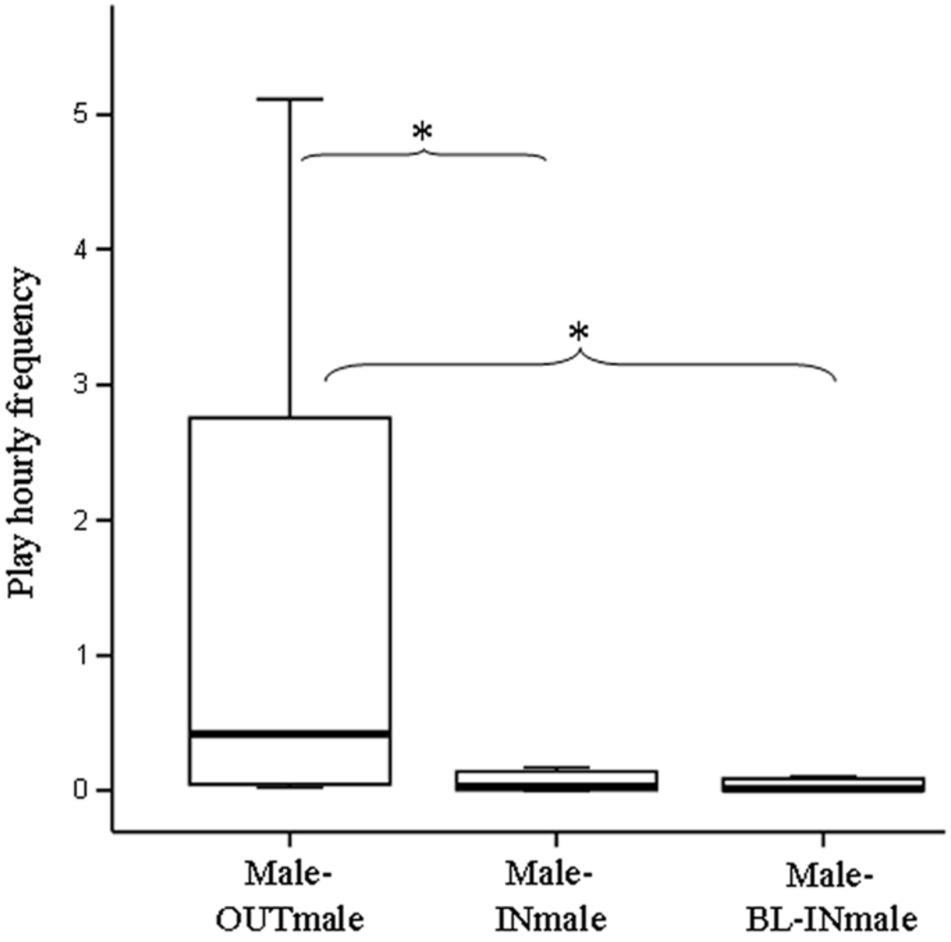

Figure 1. Levels of play between resident males and between resident and stranger males. According to the three conditions, the hourly distribution of play frequency are reported as follows: male-OUTmale (play interactions between males of the observed groups and outgroup males), male-INmale (play interactions between males of the observed groups during the visit of outgroup males), and male-BL-INmale (play interactions between males of the observed groups recorded in absence of outgroup males). Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values. Only statistically significant values are reported on the figure. The single asterisk (*) indicates $p<0.05$.
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significant difference between male-OUTmale *vs* male-INmale (male-INmale > male-OUTmale: $q = 1.98$; $p<0.05$) and male-INmale *vs* male-BL-INmale (male-INmale > male-BL-INmale: $q = 2.41$; $p<0.01$); no difference was found between male-OUTmale *vs* male-BL-INmale ($q = 0.70$; $p>0.05$) (Fig. 2a).

We compared play and grooming sessions between females and outgroup males (female-OUTmale), females and ingroup males (during the visit of outgroups, female-INmale), females and ingroup males (recorded when the outgroup males were absent, female-BL-INmale). We found no significant difference in female play distribution across the three conditions: female-OUTmale, female-INmale, and female-BL-INmale (Friedman's $\chi^2 = 1.45$; df = 2; $N_{females} = 6$; $p = 0.51$). Outgroup males and resident females initiated play sessions at comparable levels between each other (Wilcoxon's $T = 0$; ties = 3; $N_{females} = 6$; $p = 0.250$). Grooming distribution significantly differed according to the three conditions (female-OUTmale, female-INmale, female-BL-INmale) (Friedman's chi-square = 7; df = 2; $N_{females} = 6$; $p = 0.029$). In particular, Dunnett's test showed a significant difference between female-OUTmale *vs* female-INmale (female-OUTmale < female-INmale: $q = 2.12$, $p<0.01$) and female-OUTmale *vs* female-BL-INmale (female-OUTmale < female-BL-INmale: $q = 1.98$, $p<0.05$); no difference was found between female-INmale vs female-BL-INmale ($q = 1.06$; $p>0.05$) (Fig. 2b).

Aggressions and play

We compared aggression rates across three conditions: IN-OUTbefore-play (aggressions between resident and outgroup males before the first session of play), IN-OUTafter-play (aggressions between resident and outgroup males following the first session of play), and IN-IN (control variable including the aggressions between residents). Aggression rates significantly differed across the three conditions (Friedman's $\chi^2 = 8.194$; df = 2; $N_{males} = 8$; $p = 0.014$). Before play, aggression rates between resident and outgroup males were significantly higher than aggression rates between residents (IN-OUTbefore-play>IN-IN; Dunnett's test, $q = 3.54$; $p<0.01$) but such difference vanished after play (IN-OUTafter-play≈IN-IN; Dunnett's test, $q = 0.34$; $p>0.05$). Moreover, aggression rates between resident and outgroup males significantly decreased after play (IN-OUT-before-play>IN-OUTafter-play; Dunnett's test, $q = 5.79$; $p<0.01$) (Fig. 3).

We never observed any aggressive event directed by females towards outgroup males.

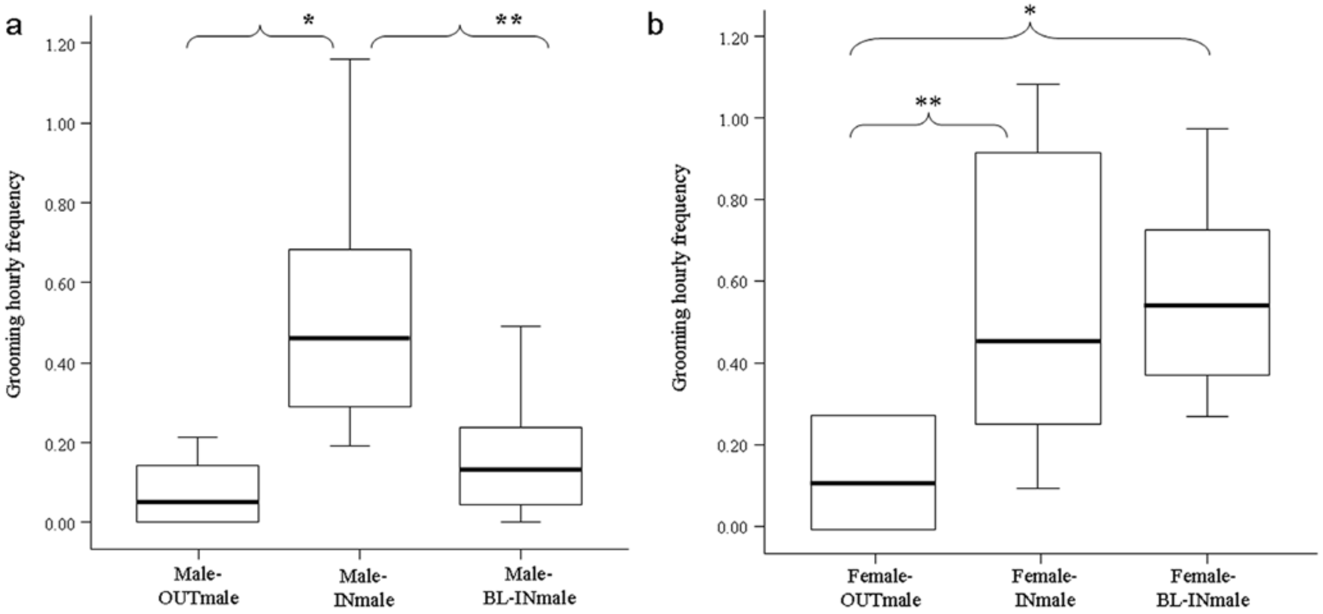

Figure 2. Levels of grooming between resident animals and between resident and stranger animals. According to the three conditions, the hourly distribution of grooming frequency is reported as follows: male-OUTmale (grooming interactions between males of the observed groups and outgroup males), male-INmale (grooming interactions between males of the observed groups during the visit of outgroup males), and male-BL-INmale (grooming interactions between males of the observed groups recorded in absence of outgroup males) (**a**). According to the three conditions, the hourly distribution of grooming frequency is reported as follows: female-OUTmale (grooming interactions between females and outgroup males), female-INmale (grooming interactions between females and ingroup males during the visit of outgroups), female-BL-INmale (grooming interactions between females and ingroup males recorded when the outgroup males were absent) (**b**). Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values. Only statistically significant values are reported on the figure. Single asterisk (*): $p<0.05$; Double asterisk (**): $p<0.01$.
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Familiarity and play intensity

We distinguished play sessions as a function of their intensity: rough (R_{play}) if the session included at least one Rough-and-Tumble pattern (as defined in Table 1; e.g. Video S1 and Video S2) and gentle (G_{play}) if not (e.g. Video S3).

G_{play} was significantly more frequent than R_{play} when play sessions involved resident males (Wilcoxon's T = 0, ties = 2; $N_{males} = 8$, p = 0.031) (Fig. 4a); on the other hand, R_{play} and G_{play} did not differ during the play sessions between resident and outgroup males (Wilcoxon's T = 11, ties = 2; $N_{males} = 8$, p = 0.938) (Fig. 4b). Consistently, the mean percentage of R_{play} frequencies was 28.54% ±SE 6.95% between resident males and 48.19% ±SE 13.12% between resident and outgroup males; whereas, the mean percentage of G_{play} frequencies was 71.46% ±SE 6.95% between resident males and 51.81% ±SE 13.12% between resident and outgroup males.

The duration of R_{play} sessions observed between ingroup males (median = 17.50, lower quartile = 3, upper quartile = 50.62) was longer than that observed between ingroup and outgroup males (median = 10.00, lower quartile = 1.25, upper quartile = 10.00) (Wilcoxon's T = 0, ties = 2, $N_{males} = 8$, p = 0.03). On the contrary, there was no difference in the duration of the G_{play} sessions (G_{play} between resident males: median = 7.50, lower quartile = 1.25, upper quartile = 10.00; G_{play} between resident and outgroup males: median = 5.00, lower quartile = 1.25, upper quartile = 10.00) (Wilcoxon's T = 2, ties = 4, $N_{males} = 8$, p = 0.50).

Discussion

The presence of unfamiliar individuals in a well-established group implies the onset of novel social circumstances, which residents have to cope with [34], [50].

This report is the first quantitative study showing that adult play can be used as the main tool for increasing tolerance and reducing xenophobic expressions between stranger animals.

Our data on wild sifaka suggest a functional dichotomy between grooming and social play (Prediction 1 supported). In fact, for both males and females play and grooming distributions do not proceed in tandem. Grooming is mostly exchanged between residents, thus being confirmed as an affinitive behavior used to maintain pre-existing social relations. The presence of outgroup individuals induces an increase of grooming between resident males (Fig. 2a). Such increase could be read as a sort of social shield adopted by resident males to consolidate and/or make their bonding more evident to the stranger "audience". This response is predicted by the xenophobia principle, which suggests that a peak of cooperative behaviors among insiders is evoked by newcomers [1]. The presence of outgroup do not seem to influence grooming distribution between resident males and females (Fig. 2b), probably because females are relieved from vigilance and resource defense, which are mostly up to males [46]. Additionally, the presence of outgroup males is an added positive value for females because it is associated to increased mate choice opportunities [39].

Whilst ingroup males engaged in play with outgroup males more than with ingroup ones (Fig. 1), females engaged in play with ingroup and outgroup males at comparable levels. Hence, male-male adult play seems not to be a purely affinitive behavior but mostly a means to test emergent relationships between strangers (Prediction 1 supported). Female-male adult play seems not to have a similar function, with outgroup males not using play to access females for courtship (Prediction 2 not supported). Female criteria for partner selection can explain such a result. To be selected by females, males have to be good scent releasers and

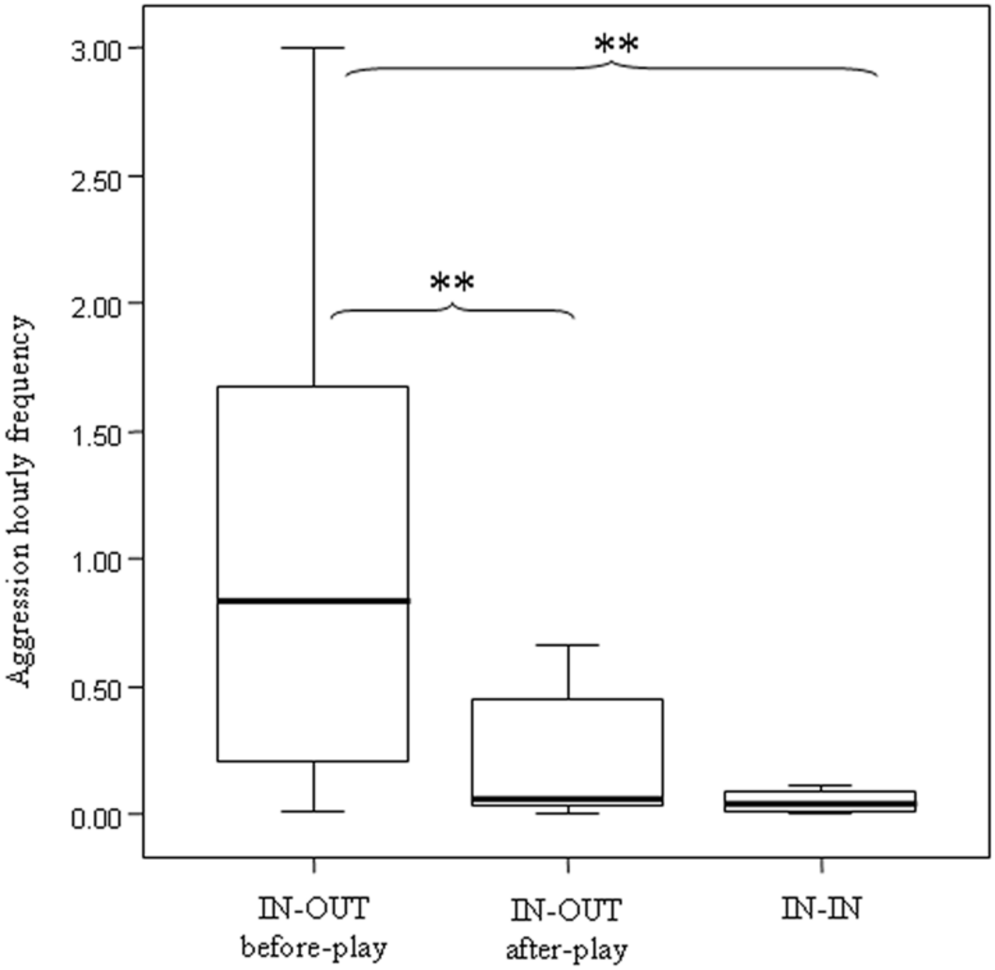

Figure 3. Levels of aggressions between resident and stranger males before and after play. According to the three conditions, the hourly distribution of aggression frequency is reported as follows: IN-OUTbefore-play (aggressions between resident and outgroup males before play), IN-OUTafter-play (aggressions between resident and outgroup males following play), and IN-IN (aggressions between residents). Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values. Only statistically significant values are reported on the figure. Single asterisk (*): $p<0.05$; Double asterisk (**): $p<0.01$.
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Table 1. Play behavioural patterns observed in adult sifaka over 481 play sessions recorded.

PLAY ITEMS	DESCRIPTION
ACROBATIC PLAY (acp)	One (solitary play) or more individuals (social play) climb, jump and dangle from supports of the environment (i.e. branches)
GRAB GENTLE (grg)	An individual gently massages the playmate
PLAY BITE (pbit)	An individual bites a part of the playmate's body
JUMP ON ANOTHER (pja)	An individual jumps with its four limbs on a playmate
PLAY PULL (ppl)	An individual grasps another playmate
PLAY PUSH (pps)	An individual pushes another playmate with its hands or feet
PLAY SLAP (psl)	An individual slaps any part of the fellow's body
PLAY BITE GENITALS (pbitg)	An individual gently bites the playmate's genitals
PLAY RETRIEVE (pre)	An individual holds the playmate in order to prevent him from leaving the play session
ROUGH AND TUMBLE (rt)	Vigorous wrestling, involving patterns such as rolling, pulling, pushing, slapping, and falling on the playmate.
GENTLE WRESTLING (gw)	Limbs entwined while sitting or laying individuals roll together placing their open mouths on each other.

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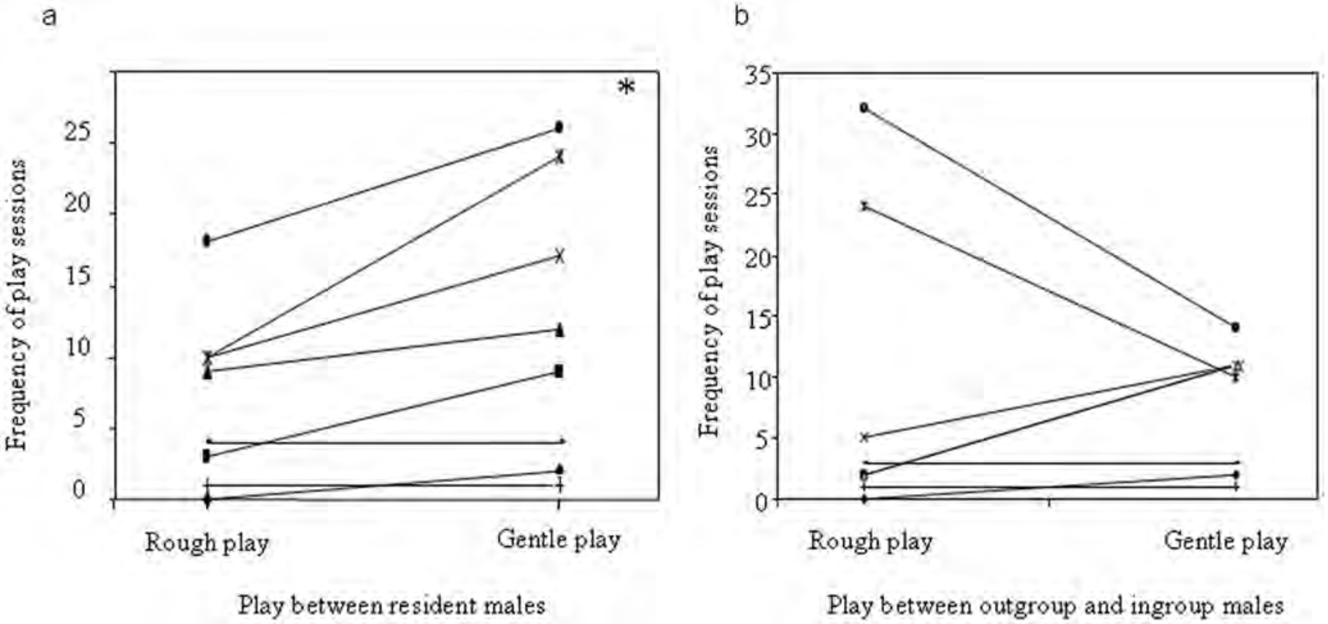

Figure 4. Differences in play modality. Rough and Gentle play interactions that occurred between resident males (ingroup-ingroup males) (**a**) and between resident and stranger males (outgroup-ingroup males) (**b**). The single asterisk (*) indicates $p<0.05$.
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groomers [39] more than good players. In fact, females grant mating priority to those males that are most active in scent-marking and a greater amount of renewed copulations to those males they receive most grooming from. In this respect, sifaka would differ from other primate species that seem to use courtship-play as a social tool for overcoming female reticence when male-female association is low (*Galago demidovii*, *Perodicticus potto*, [51]; *Mirza coquereli*, [52]; *Ateles* sp., [53]; *Pongo pygmaeus*, [54]. However, the complete lack of quantitative studies (other than the present one) on this issue leaves the role of primate play in courtship largely unexplored.

Being mostly restricted to unfamiliar males, adult play in sifaka appears to have a role in managing new social situations more than in maintaining "old" relationships. In particular, our results indicate not only that play is the interface between strangers but also that it has a specific function in reducing xenophobia (Prediction 3 supported), normally expressed by this species via aggressive chases. Aggressions by ingroup males were preferentially directed toward outgroup males more than toward other group members. After play, conflicts between unfamiliar males plunged to the levels observed between familiar males (Fig. 3). Ingroup males initiated play sessions as much as outgroup males, thus indicating that ice-breaking via play is worthwhile and beneficial for both parties. In primates, the presence of unfamiliar individuals in the group can provoke social tension and stress in animals [55]. Recent studies on rodents and primates demonstrated a link between mild stress and social play [26], [56]. For example, in rats a short period of social isolation is an effective way to increase the amount of social play when the temporary-isolated subjects are placed back with partners. In addition, experimental studies revealed that rats treated with ACTH (Adreno Cortico Tropic Hormone, a stress-related hormone) increased their play levels compared to those of saline-treated controls, thus suggesting that moderate amount of stress or anxiety promotes social play [26]. Accordingly, in order to cope with the forthcoming anxiety associated with the presence of food, captive primates increase their play levels during the time-period preceding food distribution. Moreover, dyads playing during the pre-feeding time show high levels of tolerance around food [17], [47], [57]. By helping animals to overcome stress and dissipate tension, social play in sifaka appears to represent a strategic toolkit for aggression control. This strategy is clearly advantageous because it promotes good relations between unfamiliar individuals thus reducing at minimum the costs that xenophobia would bring, in terms of aggression and group stability. This behavior has therefore immediate benefits to the animals but also long term advantages suggesting the presence in this species of cognitive capacities for anticipating future events.

We found that adult males adjust their playful tactics as a function of playmates' group membership. Since social play implies trust between individuals rough play should be more common among ingroup males than between outgroup and ingroup males (Prediction 4). Contrary to the expectations, resident males engaged mainly in gentle play sessions when playing together (Fig. 4a), whereas rough and gentle play frequencies did not differ when the play sessions involved resident and outgroup males (Fig. 4b). However, rough play sessions were longer when ingroup members only were involved (Prediction 4 partially supported). As a whole, resident males do not limit the use of the rougher mode of play when interacting with unfamiliar males, but they do limit the duration of such sessions.

Rough play is one of the most complex interactions used by animals to gather information on the potential of co-specifics as competitors or social partners [26]. In sifaka, rough play might be a sort of competitive/cooperative interaction that serves to test a partner's willingness to invest in a new relationship, and simultaneously to demonstrate one's own willingness to accept vulnerability. In short, rough play is a declaration of acceptance of the new social situation.

Rough play can be particularly risky in species which do not possess a rich repertoire of meta-communicative signals [48]. In such cases, contextual clues may be effective to avoid any misunderstanding, although what these clues are remains undetermined [58]. The use of self-handicapping, role reversal, exaggeration, and repetition also appears critical [59]. However, during particularly vigorous sessions which are consequently very risky, these subtle mechanisms may be insufficient to avoid ambiguity [55]. The short duration of rough sessions shown by resident and outgroup sifaka could be due to the lack of specific meta-communicative signals in this species [3] and, therefore, to the difficulty to maintain the playful mood.

In conclusion, our findings show that the role of play in limiting xenophobia "goes back" to the basal primate *taxon*, strepsirhines, thus revealing ancient biological roots of play in human phylogeny. In wild sifaka, play works as an ice-breaker mechanism, which enhances friendly interactions in the critical process that upgrades a stranger to a familiar individual.

Methods

Ethics statement

This study was approved by University of Pisa (Animal Care and Use board). Since the study was purely observational the committee waived the need for a permit. The study was conducted in the wild, with no manipulation of animals.

Study location

We conducted this study in the gallery forest of Berenty, a 200 ha reserve on the Mandrare River in southern Madagascar (for a complete description of the study site see [60]). In particular, this research was conducted in the northern part of the forest called Ankoba (24.99°S, 46.29°E), a 40 ha secondary forest 50–60 years old, with canopy at 10e15 m (except for a few emergent acacias to more than 20 m). The site is characterized by two main climatic periods: a wet season from October to March and a dry season from April to September [60].

Individual recognition, ingroup and outgroup animals

We observed two groups of sifaka composed of six (two adult males, one sub-adult male, two adult and one sub-adult females) and eight resident individuals (five adult males, two adult and one sub-adult females). The only infant present in one of the two groups died at the beginning of the observation period.

Non-resident adult males visited the study groups in the period around mating. Specifically, they started visiting our groups 23 days before the first mating day. We defined as "unfamiliar" nine males that were never seen with our groups in the first two months after the beginning of the observations (control period). Such males were included in the analyses as outgroup males. No outgroup female joined the group during the study period. Unfamiliar males were likely to be unrelated with most ingroup members, considering that such males mated with ingroup females, and that in *P. verreauxi* females are the phylopatric sex and group offspring is generally sired by ingroup males [61].

All resident animals were active in scent-marking, thus potentially reproductive [62]. However, lemurs undergo a transitional period in sexual maturation, indicated by a variation in the use of scent-marking (from sporadic and random to systematic) [63]. Lemurs that are not fully adult are characterized by lower marking frequencies and a smaller body size [3], which allows to be identified as subadults.

Individual recognition was based on sex and distinctive external features (scars, size, missing fur patches, fur colour, facial traits) [64]. The observational conditions (from 1 to 10 m) were excellent. In fact, animals in Berenty are well habituated to humans due to the steady presence of researchers, tourists, and local people [60].

Observational procedures

Data were collected by I.N. and E.P. in November -December 2006 and by D.A. and a field assistant from December 2006 to February 2007 (wet season).

Before starting systematic data collection, the four observers underwent a training period during which they followed the same focal animals simultaneously and then compared the data. The training (70 h of focals) was considered as completed when the observations matched in 95% of cases [65]. At the end of the training period, Cohen's kappas (k) were higher than 0.70 [66]. For each behavioral category (grooming, play, and aggressive events, as explained below) we provide the kappa range (min-max) calculated for all observer dyads (six): $k_{\text{grooming}} = 0.71–0.77$; $k_{\text{play}} = 0.74–0.81$; $k_{\text{aggression}} = 0.77–0.89$. We checked again for observer reliability in December (during one day of observation), when the second dyads of observers was about to replace the first one. Also in this case, Cohen's kappas (k) were higher than 0.70.

After the training phase, data were collected via all-occurrences sampling methods (a total of 273 hours) [67]. The observations took place daily from dawn to dusk.

Behavioral patterns

The behaviors recorded in this study were grooming, aggressions, and play.

Grooming, or fur-cleaning, in strepsirhines is typically performed via tooth-comb. For each grooming session we recorded groomer and groomee identity, grooming direction (who groomed who) and duration.

Aggressions involved agonistic encounters between individuals. For each aggression we recorded aggressor and aggressee identity, aggressive behavioral patterns (chasing, biting, and slapping); and submissive/frightened patterns (flee and vocalization).

For play behavior, we recorded initiator and receiver identity, play patterns (see Table 1), the duration of each play session, the behavioral pattern prior to each play session. A play session began when one partner directed any playful pattern (play invitation, PINV) towards a co-specific and ended when i) the playmates ceased their activities, ii) one of them moved away or iii) one of the two playmates was substituted by another individual. If the bout started again after a delay of 20 sec, it was counted as a new play session.

Operational definitions

The temporary visit of outgroup males allowed us to define three different conditions: BL-IN (interactions between resident individuals with no outgroup male present), IN (interactions between resident individuals during the visit of outgroup males), OUT (interactions between resident individuals and outgroup males). Further distinction was made on the basis of the sex of interacting individuals. Male-male interactions were labeled as male-OUTmale (interactions between males of the observed groups and outgroup males), male-INmale (interactions between males of the observed groups during the visit of outgroup males), and male-BL-INmale (control variable including the interactions between males of the observed groups recorded in absence of outgroup males). Female-male interactions were labeled as female-OUTmale (interactions between females and outgroup males), female-INmale (interactions between females and ingroup males during the visit of outgroup males), and female-BL-INmale (interactions between females and ingroup males recorded in the absence of outgroup males).

On the same observation day, we calculated aggression frequencies (bouts/hour) before and after the occurrence of the first play bout. To check whether aggression rates between resident and outgroup males decreased after engaging in a play session, we compared such rates across three conditions: IN-OUTbefore-play (aggressions between resident and outgroup males before the first session of play), IN-OUTafter-play (aggressions between resident and outgroup males following the first session of play), and IN-IN (control variable including the aggressions between residents).

Statistical analyses

The analysis was conducted at individual level. Due to the non-normal distribution of the behavioral measures (Kolmogorov-Smirnov $p < .05$), we used nonparametric statistics [68]. In order to avoid the bias due to the different number of individuals available for IN and OUT categories, all the frequencies (behavioral bouts over observation hours) recorded were normalized on the number of individuals belonging to the specific category, that is corrected for the number of potential partners.

The Wilcoxon signed-ranks test was used to compare the difference between: I) play frequencies between the outgroup males and the ingroup members; II) the frequencies of play invitation directed by the outgroup males towards ingroup members and vice versa; III) aggression rates before and after a play session occurred between resident and outgroup males; IV) play intensity (R_{play} and G_{play}) according to the group membership of males and the median duration of play sessions within ingroup males and between ingroup and outgroup males; V) the frequencies of play sessions occurring in the presence or the absence of previous social contact (sit in contact, grooming or play) according to the group membership.

We compared, by the Friedman two-way analysis of variance, play, aggression and grooming levels across three conditions: OUTmale (interactions between males of the observed groups and outgroup males), INmale (interactions between males of the observed groups during the visit of outgroup males), and BL-INmale (control variable including the interactions between males of the observed groups recorded in absence of outgroup males). The same test was used to compare play sessions between females and outgroup males (female-OUTmale), females and ingroup males (during the visit of outgroups, female-INmale), females and ingroup males (recorded when the outgroup males were absent, female-BL-INmale). In case of significant difference between the three conditions, we applied the Dunnett's multiple comparison test (post-hoc test) to determine what pairs of conditions significantly differed [68]. We used exact two-tailed tests according to Mundry and Fischer [69].

Supporting Information

Video S1 Rough play involving three males with a clear example of play fighting/rough and tumble (video by Ivan Norscia via Panasonic Lumix DMC FZ7 - 12× optical zoom/36–432 mm equivalent/Leica Lens)
Found at: doi:10.1371/journal.pone.0013218.s001 (10.09 MB AVI)

Video S2 Play sequence between males, part of longer rough session, briefly interrupted by the arrival of a third male (video by Ivan Norscia via Panasonic Lumix DMC FZ7 - 12× optical zoom/36–432 mm equivalent/Leica Lens)
Found at: doi:10.1371/journal.pone.0013218.s002 (10.19 MB AVI)

Video S3 Gentle play between resident males involving play bites (video by Daniela Antonacci via Panasonic Lumix DMC FZ7 - 12× optical zoom/36–432 mm equivalent/Leica Lens)
Found at: doi:10.1371/journal.pone.0013218.s003 (9.75 MB MOV)

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Author Contributions

Conceived and designed the experiments: IN EP. Performed the experiments: DA IN EP. Analyzed the data: DA IN EP. Contributed reagents/materials/analysis tools: EP. Wrote the paper: DA IN EP.

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Est-ce que les lémuriens bruns en liberté réconcilient? Pas toujours.

Introduction

Le comportement suivant un conflit a été largement étudiée dans les primates anthropoïdes mais très peu dans les prosimiens. Ici, nous décrivons les modalités de réconciliation d'un groupe de 14 individus de lémuriens bruns (*Eulemur rufus x collaris*) dans la forêt-galerie de Berenty (au Sud de Madagascar). Dans la période Mars-Juin 2008, nous avons récollettes les données pendant 177 heures d’observation, avec la méthode du *focal animal sampling* (échantillonnage sur des animaux focaux)

Résultats et Discussion

Nous avons trouvé que la réconciliation seulement se produit dehors du contexte d’alimentation et qu'il fonctionne en réduisant le risque que l’agression soit renouvelée. Ainsi, la réconciliation fournirait une rétroaction positive immédiate qui est probablement indépendante de la qualité de la relation entre les adversaires. La réconciliation peut aussi être un mécanisme *hic-et-nunc*, nécessaire pour éviter la diffusion de conflits dans le groupes, qui peut conduire dans les cas plus extrêmes à la rupture du milieu social.

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SHORT COMMUNICATION

Do wild brown lemurs reconcile? Not always

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Abstract Post-conflict behaviour has been widely investigated in anthropoid primates but not extensively in prosimians. Here, we report the reconciliation pattern of a 14-individual group of wild brown lemurs (*Eulemur fulvus*) in the Berenty gallery forest (South Madagascar). We found that reconciliation occurs only in the non-feeding context and that it works in reducing the risk of renewed aggression. Thus, reconciliation would provide an immediate positive feedback that is probably independent of the quality of the relationship between opponents. Reconciliation may also be a hic-et-nunc mechanism, needed to avoid conflict spreading across group members, possibly leading to social disruption.

Keywords Post-conflict behaviour · Renewed aggression · Feeding context · Madagascar · *Eulemur fulvus* · Prosimian

Introduction

Reconciliation is a form of affinitive interaction between former opponents, which make friendly contact shortly after a fight (de Waal and van Roosmalen 1979). Reconciliation functions in restoring the relationship between the opponents and the benefits associated with this (Aureli and de Waal 2000). Post-conflict reunions can also reduce the probability of further conflicts and limit stress in the victim (Aureli et al. 2002).

Few studies have focussed on post-conflict mechanisms in prosimians, all but one (Palagi et al. 2008) in captivity (Kappeler 1993; Roeder et al. 2002; Palagi et al. 2005). Malagasy prosimians, unlike anthropoids, show female dominance, lack of sexual dimorphism, and have seasonal breeding. However, they share features with anthropoids such as cohesive societies, female philopatry, and individual recognition (Wright 1999; Palagi and Dapporto 2006), which is a prerequisite for reconciliation (Aureli et al. 2002). Here, we explored reconciliation dynamics in the wild brown lemur, *Eulemur fulvus*. In particular, we examined the influence of opponents' sex class and relationship quality on conciliatory frequency and distribution. Moreover, we evaluated whether reconciliation functions in reducing the probability of renewed attacks on the victim by the same aggressor. By comparing and contrasting reconciliation profiles, it is possible to formulate hypotheses on the different degrees of social tolerance of different lemur species.

Materials and methods

Study site and group

We conducted this study at Berenty, a 200 ha reserve on the Mandrare River in Southern Madagascar. In particular, this research was conducted in the gallery forest of Malaza (S 25.00°; E 46.30°), characterized by several arboreal species and dominated by tamarinds (*Tamarindus indica*; for a complete description of the study site see Jolly et al. 2006).

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Usually, the site is characterized by two main climatic periods: a wet season from October to March and a dry season from April to September (Jolly et al. 2006).

In Berenty there is an introduced hybrid population of *Eulemur fulvus rufus* × *E. collaris* (479 individuals in 2006; Razafindramanana, personal communication).

We observed one group of *E. fulvus* comprising 14 individuals (Table 1), with clear-cut female dominance (Palagi and Norscia 2010). Kin relationships among group members were unknown with the exception of three mother–infant dyads (Table 1). The individuals were well habituated to the presence of humans. Individual identification was based on sex and on distinctive external features (scars, size, missing fur patches, fur colour, facial traits; Jolly 1972).

Data collection

The authors and three field assistants collected behavioural data from March to July 2008, a total of 177 h of actual observations, via focal animal sampling (approx. 12 h/individual). Focal observations lasted 10 min per animal and, for each individual, the focal time windows were rotated to obtain a statistical day of observation/individual at the end of the study period. *E. fulvus* is cathemeral, with 3.5 times more activity occurring with the daylight (Kappeler and Erkert 2003). Because of the need for full visibility of focal animals, observations took place in daylight, from dawn to dusk.

Table 1 Study animals, group composition

	Age/sex class	A pairs	D pairs	N pairs	CCT%
ob	AF	–	–	–	–
pen	AM	3	3	3	0.00
pal	AM	0	2	3	−25.00
ff	SF	3	1	2	33.33
mcn	AM	5	0	4	42.86
cm	AM	3	0	4	20.00
bapa	AF	2	2	4	0.00
fc	SF	2	0	6	14.29
sx	SM	3	0	7	12.50
st	SF	4	0	3	40.00
ts	AF	4	4	1	0.00
gs	IM	5	2	4	27.27
gc	IM	5	0	1	83.33
aa	IF	5	0	2	71.43
		44	14	44	26.62 ± 8.34% SE

AF adult female, *AM* adult male, *SF* subadult female, *SM* subadult male, *IF* infant female, *IM* infant male, *A pairs* attracted pairs, *D pairs* dispersed pairs, *N pairs* neutral pairs, *CCTs* conciliatory contact tendency values

We collected all agonistic interactions among individuals by use of an all-occurrence sampling method (Altmann 1974). Systematic data collection was preceded by a training period that lasted until the observations by the different observers matched in 95% of cases (Martin and Bateson 1986). For each agonistic encounter we recorded:

1. opponents;
2. context (i.e., circumstance in which the aggression took place, “feeding” and “non-feeding”);
3. type of conflict (uni-directional or bi-directional);
4. aggressive behavioural patterns (mainly chasing, biting, and slapping); and
5. submissive/frightened patterns (flee, avoid, etc.).

After the last aggressive pattern of any given agonistic event, we followed the victim (as the focal individual) for a 15 min post-conflict period (PC). Matched control observations (MCs) took place during the next possible day at the same time as the original PC, on the same focal animal, in the absence of agonistic interactions during the 15 min before the beginning of the MC and when the opponents had the possibility of interacting (within 10 m) (de Waal and Yoshihara 1983; Kappeler and van Schaik 1992; Palagi et al. 2008).

For both PCs and MCs we recorded:

1. starting time;
2. type of first affinitive interaction (contact sitting, grooming, touching, play, sexual contacts);
3. minute of first affinitive contact;
4. affinitive contact initiator; and
5. partner identity.

By focal sampling, we collected data on grooming and contact sitting to obtain information about the quality of relationships among individuals.

Data analysis

Reconciliation analysis was carried out at the individual level. For each animal we determined the number of attracted, dispersed, and neutral pairs over all PC–MC pairs. In attracted pairs, affinitive contacts occurred earlier in the PC than in the MC (or they did not occur at all in the MC), whereas in dispersed pairs the affinitive contacts occurred earlier in the MC than in the PC (or they did not occur at all in the PC). In neutral pairs, affinitive contacts occurred during the same minute in the PC and the MC, or no contact occurred in either the PC or the MC. To avoid coding the same incident twice, for each individual we used only PC–MC pairs in which that individual was the focal animal, and entered them under its name. To evaluate individual reconciliation, we used the Veenema et al. (1994) measure of conciliatory tendency (CCT), defined as "attracted minus dispersed pairs divided by the total number of PC–MC pairs". Individual CCTs were used to determine the group mean CCT.

To investigate the influence of relationship quality on reconciliation, for each individual we first calculated the mean value of affinitive interactions for dyads (grooming and contact sitting) in which that selected individual was involved. Second, for each individual we divided dyads involving it into two quality classes (weak and close) by the following procedure: dyads with both grooming and contact sitting frequencies higher than the mean value for the selected individual were assigned to the close class; alternatively, dyads with both grooming and contact sitting frequencies lower than the mean value for the selected individual were assigned to the weak class. Afterwards, we calculated the mean CCT value that each subject showed with its partners belonging to close and weak relationship quality classes. One female was never victim so it has to be excluded from analyses of post-conflict behaviour. Because of the reduced sample size ($8 \leq N \leq 13$) and non-normality of data (for $N \geq 10$, Kolmogorov–Smirnov $P < 0.05$), we applied exact non-parametric tests (Mundry and Fischer 1998). All the analyses were two-tailed ($\alpha = 0.05$). The difference in the CCT distribution according to the sex class combination (female–female, FF; male–male, MM; and female–male, FM) was evaluated at the dyadic level by randomization procedures (one-way ANOVA; 10,000 shuffles; Manly 1997) using the freeware resampling procedures 1.3 by David C. Howell.

Results

We recorded a total of 182 agonistic encounters. We could identify the opponents in 127 cases (119 decided and 8 undecided). Among adults, aggression was started by males and females with comparable levels (Exact Mann–Whitney $U = 12$; $N_f = 6$; $N_m = 5$; $P = 0.634$). Juveniles were never observed starting aggression.

We were able to collect 102 PC–MC pairs. The minimum number of PC–MC pairs recorded per focal animal was five (Table 1). Affinitive interactions between former opponents were significantly more frequent after a conflict than during control periods for the whole group (A > D: Exact Wilcoxon's $T = 2.5$, $N = 13$, $P = 0.01$) and showed a trend of significance when the analysis was restricted to adults and subadults (A > D: Exact Wilcoxon $T = 2.5$, $N = 10$, $P = 0.07$). The mean CCT was 26.62 ± 8.34% SE for all focal individuals and 20.86 ± 9.23% SE for the adults. Figure 1 shows the temporal distributions of first affinitive contacts among PC–MC.

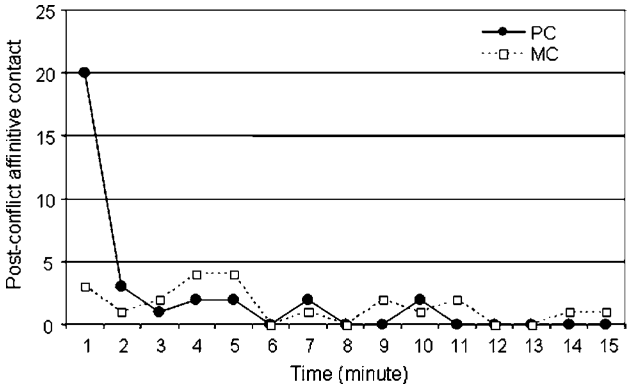

Fig. 1 Temporal distributions of first affinitive contacts in post-conflict (*PC*) and matched control (*MC*) periods for reconciliation

The first postconflict affinitive patterns used to reconcile were touching (39.4%), contact sitting (33.3%), and grooming (27.3%). The use of such patterns in the reconciliation process did not differ significantly (Exact Friedman's test $\chi_r^2 = 0.70$, $df = 2$, $N = 12$, $P = 0.73$).

According to different aggression contexts (feeding and non-feeding) we found that attracted and dispersed pairs did not significantly differ in the feeding context (exact Wilcoxon signed-rank test $T = 18$, $N = 13$, $P = 0.71$) but they differed in the non-feeding context (attracted pairs > dispersed pairs; exact Wilcoxon signed-rank test $T = 0$, $N = 9$, $P = 0.004$, mean CCT = 44.45 ± 2.78% SE). Only 9 individuals were available for the non-feeding context analysis.

Males and females showed comparable CCT levels (exact Mann–Whitney $U = 20$, $N_{\text{males}} = 7$, $N_{\text{females}} = 6$, $P = 0.943$). At the dyadic level, we found no difference in the distribution of CCTs according to opponents' sex (randomization one-way ANOVA $F = 1.126$, $N_{\text{ff}} = 13$, $N_{\text{mm}} = 14$, $N_{\text{mf}} = 23$, $P = 0.332$).

The occurrence of conciliatory contact significantly reduced the probability of renewed aggressions on the victim (exact Wilcoxon signed-rank test $T = 0$, $N = 13$, $P = 0.031$). Renewed aggression accounted for 48.11 ± 9.44% SE in the absence of reconciliation and 18.25 ± 8.10% SE in its presence.

We found no difference in the CCT levels according to close and weak relationships (exact Wilcoxon signed-rank test $T = 10$, $N = 8$, $P = 0.578$).

Discussion

Reconciliation was present in the wild group of *E. fulvus,* thus confirming previous results in captivity (Kappeler 1993; Roeder et al. 2002). Reconciliation has also been found in wild *Propithecus verreauxi* (Palagi et al. 2008), but not in captive *Eulemur macaco* or in three out of the four captive groups of *Lemur catta* where post-conflict reunions were investigated (Kappeler 1993; Roeder et al.

2002, Palagi et al. 2005). Although all species have a similar social organisation with female dominance, *E. fulvus* and *Propithecus verreauxi* are characterized by more tolerant intra-group relationships than *L. catta* and *Eulemur macaco* (Kappeler 1993; Roeder et al. 2002; Palagi et al. 2008; Palagi and Norscia 2010). Thus, the level of despotism in the social group, more than the phylogenetic closeness or the social organisation, seems to account for the presence of reconciliation in social prosimians.

As a form of direct food competition, contests over food can be particularly risky (Ricklefs 2001; van Schaik and van Noordwijk 1988), thus limiting post-conflict reunions (Aureli 1992). Moreover, affiliations are unlikely because animals are busy eating or searching for food. Consistently, in *E. fulvus*, reconciliation turned out to be present in the non feeding context but not during feeding sessions. Similar findings have been already reported in other primates (e.g. *Propithecus verreauxi*: Palagi et al. 2008; *Cebus apella*: Verbeek and de Waal 1997; *Macaca* spp.: Majolo 2004, Aureli 1992, Matsumura 1996).

In female philopatric species, for example lemurid ones, females have more interest in establishing long-term relationships with other females than males (Silk et al. 2003). Thus, females should be more involved in conflict resolution than males. However, in *E. fulvus* (this study), and in *Propithecus verreauxi* (Palagi et al. 2008) reconciliation levels did not vary as a function of the sex class combination of the former opponents. This result could be linked to the fact that both studies included the mating period, when male–female bonding increases as a result of the biological market effect (Norscia et al. 2009).

Conciliatory contacts significantly reduced the probability of renewed attacks (particularly frequent in the study group) on the victims by former aggressors. This result is consistent with the finding that in this species, and in anthropoids (Kutsukake and Castles 2001) reconciliation works in reducing victims' post-conflict stress (Palagi and Norscia 2010), probably related to the reduced risk of being re-attacked. In this perspective, reconciliation would provide an immediate positive feedback that is probably independent of the relationship quality (weak/close) that the opponents have "built" over time.

A broader, and not exclusive, hypothesis is that reconciliation, in this species, works as a hic-et-nunc mechanism (that is producing immediate benefits), needed to settle hostilities and avoid conflict spreading across group members, possibly leading to social disruption. Further investigation on with a larger sample size (in terms of both groups and animals) is certainly required to address this issue and draw final conclusions.

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Stress (2011) 14 : 93-97

Se gratter tout autour du stress: hiérarchie et réconciliation font la différence chez les lémuriens bruns en liberté (*Eulemur fulvus*).

Introduction

Le lien entre grattage (*scratching*) et stress a été démontré chez les singes mais jamais dans les prosimiens, soit dans la nature ou en captivité. Norus avons analysé les données recueillies sur un groupe de 14 animaux de *Eulemur rufus* x *collaris* dans la forêt de Berenty (Sud de Madagascar, Mars-juillet 2008). Nous avons appliqué un protocole (mêmes conditions météorologiques, intervalle de temps, contexte social/activité, area de forêt, et la formation de sous-groupes) impliquant quatre conditions, en vertu de las quelles nous avons enregistré le comportement de grattage: tentative de prédation, conflits réconciliés, conflit non réconciliés, et condition de contrôle.

Résultats et Discussion

Nous avons constaté que le lien entre grattage et stress reste existe aussi dans les prosimiens. Le comportement de grattage augmentait après les attaques prédatrices par le faucon *Polyboroides radiatus* et après agressions intra-groupe. Par contre, le comportement diminuait après la réconciliation, probablement parce que la réconciliation réduit le stress qui suive le conflit. Nous avons observé une corrélation négative entre le comportement de grattage et la hiérarchie, qui est linéaire, mais seulement en absence d'événements stressants. Dans cette condition les individus dominants apparaissait moins stressés que les subordonnais. Comparé aux agressions, les tentatives de prédation induisait une augmentation plus grande du niveau grattage, et les dominants montrait l'augmentation plus élevé. Ainsi, le grattage est sensible aux différents types de perturbation de l'homéostasie (prédation ou agression) et n’est pas seulement un mécanisme *tout ou rien*. Après un cadre théorique basé sur des analyses de cortisol, nous avons montré les données hormonaux convergent avec les résultats comportementaux en indiquant que le profil de stress d'une espèce dépend des caractéristiques de son réseau sociales.

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Scratching around stress: Hierarchy and reconciliation make the difference in wild brown lemurs (*Eulemur fulvus*)

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Abstract

The scratching–stress linkage has been demonstrated in monkeys and apes but never in strepsirrhines, either in the wild or in captivity. We analysed data collected on a 14-animal group of *Eulemur fulvus* in the Berenty forest (South Madagascar, March–July 2008). We applied a protocol (same weather conditions, time slot, social/activity context, forest quadrat, and subgroup formation) involving four conditions, under which we recorded the scratching response: predation attempt, reconciled conflict, non-reconciled conflict, and control. We found that the scratching–stress linkage remains valid in strepsirrhines. Scratching increased after predatory attacks by the hawk *Polyboroides radiatus* and intra-group aggressions and decreased after reconciliation, probably buffering post-conflict stress. Scratching negatively correlated with the linear hierarchy, but only in the absence of stressful events. Compared to aggressions, predation attempts induced a greater increase in scratching, with dominants showing the highest differential increase. Thus, scratching is sensitive to different kinds of homeostasis perturbation (predation/aggression) and does not simply provide all-or-nothing information. Following a theoretical framework based on previous cortisol analyses, we showed that scratching and hormonal data converge in indicating that the stress profile of a species is shaped by its social network features.

Keywords: *Aggression,* Eulemur fulvus*, lemurs, predation, ranking position, self-directed behaviours*

Introduction

In humans, non-verbal behaviours are an integral part of how emotions are expressed (Darwin 1872), which include stress related to fear, deception, motivational conflict, uncertainty in decision making, or resolution of difficult tasks. In other primates, some of these emotional states have been proved to be stress inducers, as well. Stress is a physiological response to events perceived as potentially or actually threatening the integrity of the body (Sapolsky 2005).

Ethological observations associated to pharmacological trials have clearly shown the tight linkage between stress-induced hormones (plasma cortisol) and self-directed behaviours (mainly self-scratching) in monkeys (Troisi 2002). Studies on group-living monkeys revealed that self-scratching is sensitive to pharmacological manipulation of mood through anxiolytic and anxiogenic substances (Schino et al. 1996). In wild monkeys and apes, self-scratching represents a non-invasive means to detect stress, which can be increased by different socio-ecological factors, including predatory attacks and intra-group aggressions (Castles et al. 1999; Kutsukake 2003). Moreover, within social groups the relative hierarchical position of individuals affects stress levels and reflects on baseline self-scratching frequencies (with either dominants scratching more than subordinates or vice versa). The scratching trend across hierarchy depends on social organization in different species or populations, with subordinates showing the highest stress levels in hierarchically stable social groups (Sapolsky 2005).

Owing to their cryptic lifestyle (mainly linked to nocturnality and dispersed sociality), strepsirrhine primates have been previously neglected in this field of research. Within strepsirrhines, group-living lemurs make an exception because they share basic features with anthropoids such as diurnal habits and cohesive

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multimale/multifemale societies (Pereira and Kappeler 1997).

Although scratching has already been used as a stress indicator in lemurs (Nunn and Deaner 2004), here we evaluated for the first time in lemurs if scratching is, indeed, a reliable indicator for quantifying stress and if post-conflict (PC) affiliation buffers the stress related to aggression as reported for other primates (Aureli et al. 2002; Fraser et al. 2010). As a study species, we selected *Eulemur fulvus* (the brown lemur) living in the Berenty forest (South Madagascar). If scratching functions as a fine-tuned indicator of stress, we expected it to increase differentially following events (predatory attacks and aggressions) that produce different stress responses. We also expected that scratching variation anticipates the information provided by the theoretical model based on hormonal data (Abbott et al. 2003), related to the social network features of *E. fulvus*.

Material and methods

Data collection

We conducted the study in the Berenty gallery forest (South Madagascar; S 25.00°; E 46.30°) from March to July 2008. We observed a 14-individual group of introduced *E. fulvus rufus* × *collaris* (brown lemurs) (Table I). The animals, which could see the observers and were habituated to their presence, were sexed and individually identified via facial–body features. *E. fulvus* is cathemeral, with 3.5 times more activity occurring during daylight (Kappeler and Erkert 2003).

We applied a protocol (same weather conditions, time slot, social/activity context, forest quadrat, and subgroup formation) involving data collection in four conditions, under which we recorded the scratching response: predation attempt, reconciled conflict (RC), non reconciled conflict (NRC), and control (MC). The predation attempt context refers to 15-min observations after predation attempts by *Polyboroides radiatus* (Madagascar Harrier-hawk), the only lemur predator present in the area. Predation attempts include hawks flying overhead and inducing lemurs to produce an alarm call and/or a flee response. The non-reconciled context refers to conflicts, either not yet reconciled or not reconciled at all. A conflict was reconciled when former opponents engaged in an affinitive contact within 15 min following the aggression (PC period).

Table I. The brown lemur group under study.

Animal label	Age/sex class	Ranking position
TS	Adult female	1
BAPA	Adult female	2
OB	Adult female	3
PAL	Adult male	4
PEN	Adult male	5
CM	Adult male	6
SX	Subadult male	7
ST	subadult female	8
FF	Subadult female	9
FC	Subadult female	10
MCN	Adult male	11
AA	Late-infant female, TS's daughter	12
GS	Late-infant male, BAPA's son	13
GC	Late-infant male, OB's son	14

With daylight, the authors and three assistants collected 177 h of behavioural data in real time via direct observations (Altmann 1974). Through focal sampling (about 12 h/individual) we collected data on self-scratching, hereafter termed scratching (fur rubbing via finger toilet-claws). Through all occurrences, we collected data on agonistic encounters and recorded (i) opponents, (ii) conflict type (decided, with a clear winner, or undecided), and (iii) aggressive (chasing, biting, and slapping) and submissive/frightened patterns (flee, avoid, and vocalizations).

After the last aggressive pattern of any agonistic event, we observed the victim for a PC and matched control (MC) periods (de Waal and Yoshihara 1983), each lasting 15 min. For both PCs and MCs, we recorded starting time, minute, initiator, and first affinitive contact. At the beginning of MCs, the opponents were a maximum of 10 m apart.

Definitions and statistics

For each lemur, we determined the number of attracted (*A*), dispersed (*D*), and neutral (*N*) pairs over all PC–MCs (de Waal and Yoshihara 1983). To avoid coding the same incident twice, for each individual we used only PC–MC pairs in which that individual was the focal animal and entered them under its name. As one female was never a victim, it was excluded from the analyses.

We used Wilcoxon's test to check for the presence of reconciliation (*A* vs. *D* pairs) and Friedman's test to check for the differences among the scratching levels in the different conditions (predation attempt, RC, NRC and MC). We applied Dunnett's test for *post hoc* analyses.

Through Spearman's test we assessed the correlation between (i) scratching and ranking in predation attempt, NRC, and MC and (ii) scratching variation index (SVI, see Figure 2b) and ranking positions. The individual SVI was calculated as the difference between scratching levels under stressful (SCR_{STR}) and baseline conditions (SCR_{MC}) over the total scratching levels. Scratching levels in a non-reconciled context and predation attempt context were tested separately.

Through SPSS 12.0 and STATXACT 7.0, we performed exact two-tailed analyses ($\alpha = 0.05$; Mundry and Fischer 1998). We used dyadic decided conflicts to test for hierarchy linearity (MatMan 1.0; de Vries et al. 1993).

Background analysis

We recorded 182 conflicts. We could identify the opponents in 119 decided conflicts. Victims never received support by conspecifics. For the first time, we found a linear hierarchy in *E. fulvus*, considering both the whole group ($h' = 0.48$; $n = 14$; $DC = 0.76$; $p = 0.009$) and the adults only ($h' = 0.51$; $n = 11$; $DC = 0.67$; $p = 0.039$). All the adult females were at the top of the reordered matrix fitting linear hierarchy (Table I). Hierarchy linearity, demonstrated here for the first time, allowed the assessment of the scratching–ranking relationship (i.e. ranking the hierarchical position).

We collected 102 PC–MCs (five PC–MCs per individual minimum). Affinitive interactions (body contact, grooming, touching, play, and sexual contacts) between former opponents were significantly more frequent in PCs than in MCs ($A > D$: $T = 2.5$; $n = 13$; $p = 0.010$). A total of 62.5% of conciliatory contacts occurred within the first PC minute.

Results

Scratching levels significantly differed across predation attempt, NRC, and MC ($\chi_r^2 = 12.70$; $n = 13$; $p = 0.001$). *Post hoc* tests confirmed the significance for each variable pair: predation attempt/MC ($q = 3.38$; $p < 0.01$), NRC/MC ($q = 2.60$; $p < 0.01$),

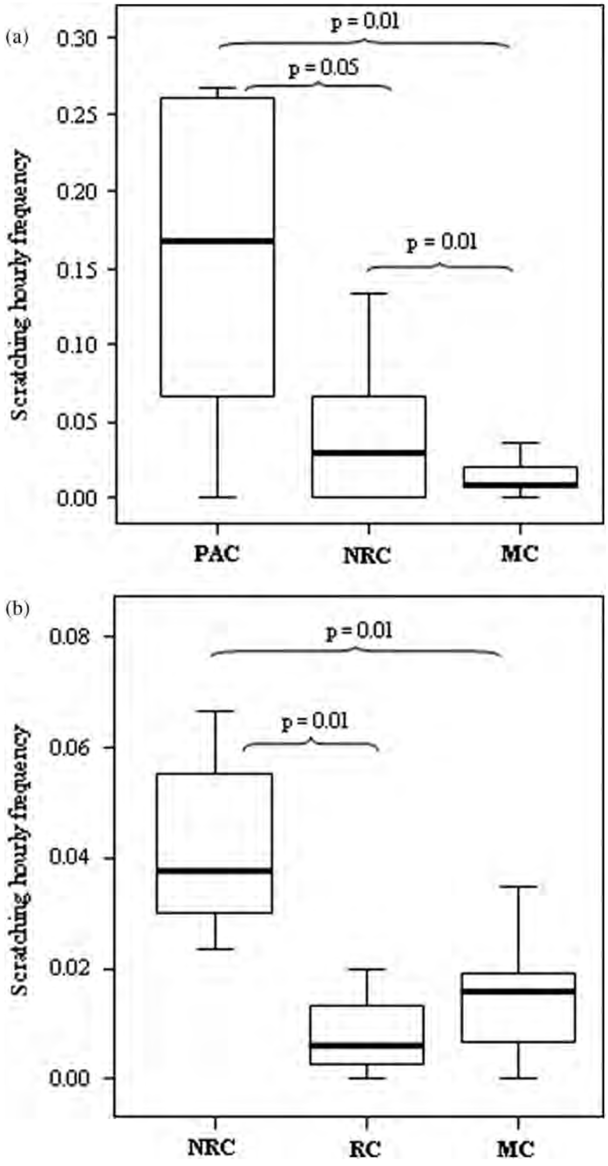

Figure 1. Scratching frequency (by 13 *E. fulvus* individuals) in (a) predation attempt (PAC), non-reconciled condition (NRC), and MC and (b) after conflict in NRC and reconciled condition (RC) and MC. Solid horizontal lines indicate medians, box length corresponds to the interquartile range, and thin horizontal lines indicate the observed value range. p values are from Dunnett's *post hoc* tests.

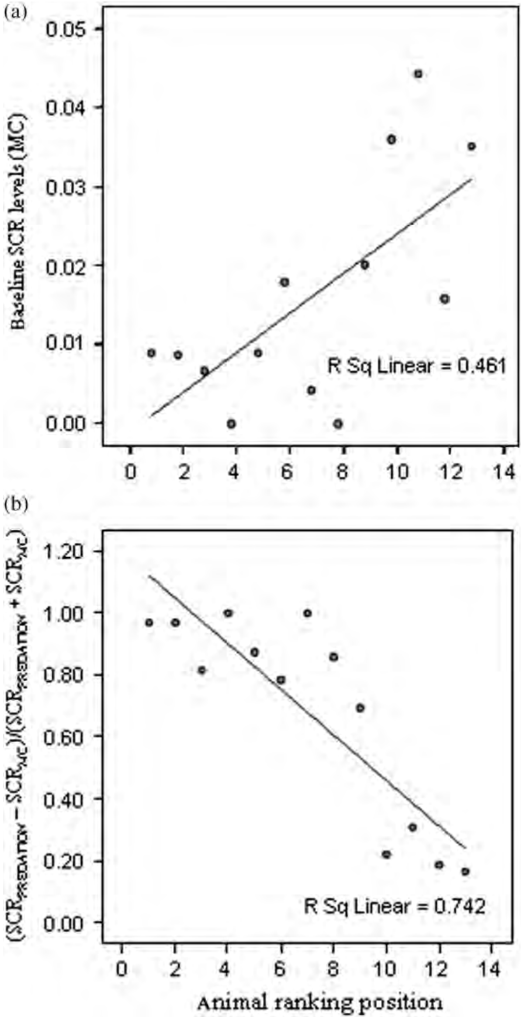

Figure 2. Scatter plots showing (a) the positive Spearman's correlation between baseline scratching (SCR_{MC}) and rank and (b) the negative Spearman's correlation between the SVI following a predation attempt ($SVI_{PREDATION}$ defined in y-axis) and rank. $N = 13$ individuals.

and NRC/predation attempt ($q = 2.18$; $p < 0.05$) (Figure 1a). Scratching levels differed significantly across NRC, RC, and MC ($\chi_r^2 = 8.86$; $n = 10$; $p = 0.008$) (Figure 1b). Only 10 individuals were available for all these three conditions. The significance arose from two variable pairs: NRC/MC ($q = 2.64$; $p < 0.01$) and NRC/RC ($q = 2.91$; $p < 0.01$), but not from the RC/MC pair ($q = 1.50$; $p > 0.05$).

Ranking position and scratching negatively correlated in MC ($r_s = -0.601$; $n = 13$; $p = 0.030$) (Figure 2a) but not in NRC ($r_s = -0.088$; $n = 13$; $p = 0.774$) and predation attempt ($r_s = 0.522$; $n = 13$; $p = 0.10$). Individual SVI and ranking position positively correlated in predation attempt ($r_s = 0.801$; $n = 13$; $p = 0.001$) (Figure 2b) but not in NRC ($r_s = 0.063$; $n = 13$; $p = 0.837$).

Discussion

Our study provides the first evidence for a linkage between stress and scratching in strepsirrhines.

In *E. fulvus*, the increase of stress-related behaviour above baseline levels observed after a predation attempt is obviously related to life risk: a predatory attack is always a source of acute stress, inducing the typical fight-or-flight response, part of a general adaptation syndrome (Sapolsky 1990).

The increase in the scratching levels following a conflict is possibly due to an uncertainty in decision making: withdrawing for fear of renewed attacks and approaching to reconcile (Aureli 1997).

Both predation attempt and aggression resulted in increased scratching but the response was higher in the first case. Thus, the change in scratching levels (as well as cortisol variation; Sapolsky 1990) is not an all-or-nothing response to stress. Whilst the scratching variation itself is considered to indicate a change in the animals' emotional state, the entity of such variation may be considered to provide an estimate of the relative amount of the stress accumulated after different events.

In wild brown lemurs (a) baseline scratching decreased as animal's ranking position increased (Figure 2a); (b) such negative correlation vanished when the analysis was restricted to the scratching performed by victims after predation attempts and aggressions and (c) dominants showed the differential increase in scratching after predation attempts, as revealed by the positive correlation between rank and SVI (Figure 2b).

Following a theoretical framework based on previous plasma/salivary cortisol analyses, it is possible to predict that (a) subordinates will exhibit the highest baseline stress levels in case of social stability and when experiencing higher rates of social stressors and lower social support (Abbott et al. 2003), (b) dominants and subordinates will be affected to a similar extent by social uncertainty, which in group-living mammals is an additional stressor inducing an increase in corticosteroid concentrations (Sapolsky 1990, Alexander and Irvine 1998), and (c) dominants, even if characterized by the lowest baseline stress levels, will show the highest stress increase (linked to adrenocortical reactivity) when exposed to ecological/environmental stressors (Manogue et al. 1975; Hellhammer et al. 1997).

The scratching behaviour of brown lemurs and the hormonal data from studies on other primate groups draw a similar stress profile shaped by specific social network features. In particular, (a) linear hierarchy informs social stability (Sapolsky 1992; Abbott et al. 2003) and subordinate lemurs experience the highest social stress (being continuously threatened by dominants via agonistic displays and aggressions) and the lowest coalitionary support (mostly supplied to dominants) (Pereira and Kappeler 1997); (b) social unpredictability is a "blind" agent acting on the stress levels of both dominants (especially if it implies a rise or decline in the hierarchy; Sapolsky 1992) and subordinates (especially due to the risk of renewed attacks; Aureli et al. 2002), and (c) predation attempts expose lemurs not to a social stressor but to an environmental one, which affects dominants preferentially. Alternatively, subordinate individuals would show a lower increase in scratching because their baseline levels are higher and do not allow an additional large increase in scratching (probably reflecting a physiological constraint). By contrast, aggression levels, higher in dominants, are positively correlated to testosterone levels (Challenge hypothesis; Archer 2006), which, in turn, seem to be negatively linked to stress hormone levels, at least in humans (e.g. cortisol; Daly et al. 2005). Thus, dominants would show lower baseline stress levels, reflecting in a higher differential response to acute stressors.

Finally, reconciliation (not investigated in the wild for this species but already described in captivity by Kappeler (1993)) seems to reduce PC stress. Indeed, after reconciliation, victims' scratching decreased to the baseline levels. Others have demonstrated this function in monkeys and apes (Aureli 1997; Fraser et al. 2010), but not in strepsirrhines, neither in the wild nor in captivity.

This study shows, for the first time, that the most basic primate group (strepsirrhines) does not make an exception in the primate world with respect to behavioural response modulation following stressful conditions. Moreover, the scratching profile of lemurs fits with the theoretical framework based on hormonal data from non-strepsirrhine primates. Thus, in an evolutionary perspective, scratching, as a stress response, may have been maintained throughout primate evolution. Seasonal changes in the baseline stress levels and hormonal data from strepsirrhines should be considered for future investigations.

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Qualité des fragments et la distribution du primate arboricole *Propithecus verreauxi* dans la forêt épineuse du Sud Madagascar

Introduction

La fragmentation d'une forêt peut provoquer un distribution aléatoire des animaux parmi les différentes parcelles la matrice entourant les fragments. Les populations locales ne peut survivre que si les restes de forêt colonisés sont adéquats et/ou la dispersion vers des autres fragments est possible. Une répartition non aléatoire peut être le résultat de différentes populations locales qui restent connectés, mais distincte (métapopulations) ou de la fusion de surpopulations qui forment une seule population, plus grande mais fragmentée. Cette répartition non casuel peut être dictée par des différents aspects de la qualité de fragments, y compris la dimension et de la variabilité de la végétation (par exemple la diversité des espèces, l'abondance de grands arbres et la disponibilité des plantes comestibles).

Résultats et Discussion

Cette étude évalue l'état de la population et de la distribution du primate malgache *Propithecus verreauxi* en six fragments de la forêt épineuse du sud de Madagascar. *Propithecus verreauxi* (le sifaka de Verreaux) est un lémurien particulièrement sensibles à la perturbation de l'habitat, car il est strictement arboricole (grimpeur verticale et sauteur) et a un régime alimentaire qui fournit peu d'énergie (il est principalement folivore). Nous rapportons ici les résultats du recensement et de la collecte de données relatives aux six fragments considérés.

Fragment	**Area (hectares)**	**Distance de la fleuve (Mandrare)**	**Nombre de lémuriens (*P. verreauxi*)**	**Proportion d'arbres avec un DHP*≥5cm**	**Richesse en termes de morphoespèces de plants**	**Habitat autour des fragments**
Partie épineuse de Malaza	4.9	0.56	6	0.61	23	Pâturage, broussailles
Reserve épineuse Berenty 1	27.8	1.16	16	0.66	23	Champs de sisal, pâturage
Reserve épineuse Berenty 2	10.6	2.2	13	0.66	24	Champs de sisal, pâturage
Rapily (ouest)	11.3	2.47	3	0.42	28	Champs de sisal, forêt épineuse dégradée
Fragment X	3.1	1.6	4	0.56	22	Champs de sisal
Anjapolo	76.0	1.36	7	0.61	24	Champs de sisal, pâturage

*Diamètre à hauteur de poitrine

Nos résultats suggèrent que les facteurs intrinsèques de la population (densité et sex-ratio) n'expliquent pas la viabilité de la population et que, parmi les facteurs extrinsèques, la structure de la végétation semble être plus important de la taille des fragments à dicter la répartition des animaux dans les petits fragments de forêt épineuse.

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SHORT COMMUNICATION

Fragment quality and distribution of the arboreal primate *Propithecus verreauxi* in the spiny forest of south Madagascar

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Key Words: Berenty, Didieraceae, fragmentation, lemur, Madagascar, prosimian, sifaka

The increasing proportion of the landscape used by humans has led, and is still leading, to the conversion of the original habitat into numerous small patches, often separated by a matrix of inhospitable land-uses. This habitat fragmentation is a major threat to biological diversity and is considered to be the primary cause of the present species extinction crisis (Aurambout *et al.* 2005). Survival in fragments is related to both intrinsic factors, such as abundance and sex ratio, and extrinsic factors related to patch quality (Ramanamanjato & Ganzhorn 2001, Rovero & Struhsaker 2007). At first, the fragmentation process can randomly distribute animals among forest patches and across fragmented habitat and surrounding matrix (Marsh 2003, Tischendorf *et al.* 2005). Local populations can survive only if the colonized forest remnants are adequate and/or dispersal is possible (Marsh 2003). Subsequently, a non-random distribution can result from local populations either remaining connected but distinct (metapopulation) or merging into a single large but patchy population (Harrison & Taylor 1997). Such distribution can be dictated by different aspects of fragment quality, including size and vegetation variables (e.g. tree species diversity, large-tree abundance and food plant availability) (Ramanamanjato & Ganzhorn 2001, Rovero & Struhsaker 2007). The mutual relationship among variables and their linkage to animal abundance have proven difficult to disentangle and mammals largely diverge in their response to different fragment quality aspects (Irwin 2008, Ramanamanjato & Ganzhorn 2001, Rovero & Struhsaker 2007).

This study evaluates, for the first time, population status and distribution of the Malagasy primate *Propithecus verreauxi* (Grandidier 1867) in spiny forest fragments of south Madagascar. *Propithecus verreauxi* (Verreaux's sifaka) is a lemur particularly sensitive to habitat disruption because it is strictly arboreal (vertical climber and leaper) and has an energetically poor diet (mainly folivorous; Norscia *et al.* 2006).

In this study we checked for possible intrinsic and extrinsic factors influencing sifaka distribution in spiny forest fragments, also compared to the riverine forests of the same area.

In March–April 2008 the authors and two field assistants performed a sifaka survey in the Berenty Estate (Androy Region; rainfall averages less than 500 mm y^{-1}). The survey covered 134 ha of spiny forest and 60 ha of riverine forests. The spiny forest is usually 3–6 m in height with dwarf and xerophytic plants, and emergent trees (up to more than 10 m) of the Didieraceae, dominated by *Allouadia procera* Drake (Elmqvist *et al.* 2007).

We performed a first survey in those spiny-forest fragments (24.93–25.03°S; 46.21–46.31E; Table 1; Figure 1) that are not used as cemeteries, which can be only accessed by local Tandroy family clans. All fragments underwent divisive fragmentation except one (Spiny Malaza), which probably underwent regressive fragmentation (*sensu* Marsh 2003) (Table 1).

We performed a second survey in three non-spiny areas of the Berenty Reserve, on the Mandrare river, comprising a northern section (the 40-ha secondary forest of Ankoba dominated by the exotic species *Pithecellobium dulce* (Roxb.) Benth.; 24.99°S, 46.29°E) and a southern section (Malaza: 25.01°S, 46.31°E), including a 7-ha

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Table 1. Study fragments and variables: Area (ha); DFR: distance from the river (m); SA: sifaka abundance (number of individuals); LTP: proportion of trees with dbh $\geq$ 5 cm; PMR: plant morphospecies richness (number of morphospecies).

Fragment	Area	DFR	SA	LTP	PMR	Surrounding matrix
Spiny Malaza	4.9	0.56	6	0.61	23	Pasture, scrub
Spiny reserve 1	27.8	1.16	16	0.66	23	Sisal fields, pasture
Spiny reserve 2	10.6	2.2	13	0.66	24	Sisal fields, pasture
West rapily	11.3	2.47	3	0.42	28	Sisal fields, degraded spiny forest
Fragment X	3.1	1.6	4	0.56	22	Sisal fields
Anjapolo	76.0	1.36	7	0.61	24	Sisal fields, pasture

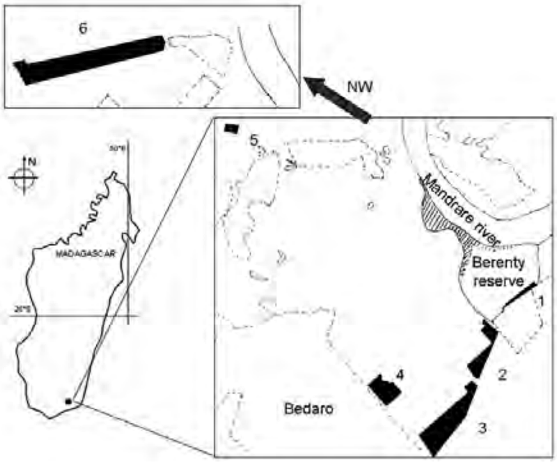

Figure 1. Study site location: Berenty reserve (solid outline; white area: scrub; diagonal lines: Ankoba and Malaza riverine forests) and spiny forest fragments (black areas): 1 = Spiny Malaza, 2 = Spiny Reserve 1; 3 = Spiny Reserve 2; 4 = West Rapily; 5 = Fragment X; 6 = Anjapolo, about 13 km north-west of Berenty. Dashed outlines include degraded spiny and/or scrub areas. The rest of the territory (white) is covered by pasture and sisal fields. (Map based on Google Earth satellite view.)

gallery forest (dominated by tamarinds; *Tamarindus* indica L.) and a transitional forest (called 'front'; 13 ha) between the gallery forest and a scrub area (Jolly *et al.* 2006) (Figure 1). In all areas logging and hunting are prohibited, the fossa (*Cryptoprocta ferox* Bennett 1833) is absent and, consequently, predation pressure is low. The minimum distance of each forest site from Mandrare river was evaluated via Google Earth.

We counted and sexed sifaka by walking at a speed of about 1 km h^{-1} along trails and forest paths chosen ad hoc to have visibility of at least 50 m right and left, cover the whole area and to avoid pseudoreplication (Norscia & Palagi 2008). For each forest area, the sex ratio (proportion of females to males) was calculated when at least one complete animal group could be sexed (fragment X was excluded).

In each fragment, we gathered vegetation data in an area of 0.1 ha divided into two subunits of 0.5 ha. With a local botanist we identified and counted plant morphospecies, and measured tree dbh (diameter at breast height), an indicator of leaf availability (Elmqvist *et al.* 2007, Ganzhorn 1995). We considered trees with a dbh $\geq$ 1 cm, thus excluding seedlings and including saplings (dbh 1–4.9 cm) and medium/large trees (dbh $\geq$ 5 cm).

Owing to the small sample size ($n < 10$ for forest sites) or deviation from normality (when $n \geq 10$, for groups; Kolmogorov–Smirnov, $P < 0.05$), we applied non-parametric exact tests (software: SPSS 12.0 and STAXACT 7.0). For multiple tests, significance ($\alpha = 0.05$) was adjusted downward via Bonferroni technique and $P < 0.05$ considered as a trend.

In total we counted 183 sifaka adults and 25 infants (less than 1 y old, not included in the analyses). Sifaka density (number of individuals/fragment area) and the minimum distance of forest sites from the river (0–0.16 km for the riverine forests; 0.56–2.47 for the spiny forest fragments, Table 1) were negatively correlated (Spearman, $n_{\text{forest sites}} = 9$, $r = -0.695$, $P < 0.05$). Consistently, sifaka density was higher in riverine forest areas (range: 1.80–3.24 ind. ha^{-1}; mean ± SD = 2.44 ± 0.73 ind. ha^{-1}) than in spiny-forest fragments (range = 0.09–1.31 ind. ha^{-1}; mean ± SD: 0.78 ± 0.54 ind. ha^{-1}) (Mann–Whitney U test, $n_{\text{riverine}} = 3$, $n_{\text{spiny}} = 6$, $Z = -2.32$, $P < 0.05$) (Figure 2, Table 1).

In riverine forest areas we counted 81 adult males and 57 adult females (adult sex ratio: 0.70), with males significantly outnumbering females (Wilcoxon test: $n_{\text{groups}} = 32$, ties = 10; $T = 19$, $P = 0.001$). In the spiny forest we counted 45 individuals but we were able to sex 21 adult males and 19 adult females (adult sex ratio: 0.90); males did not significantly outnumber females (Wilcoxon test: $n_{\text{groups}} = 9$, ties = 4; $T = 3$, ns). Overall, sifaka sex ratio was significantly more skewed in the non-spiny-forest areas than in the spiny-forest fragments (Mann–Whitney U test, $n_{\text{non-spiny}} = 3$, $n_{\text{spiny}} = 5$, $Z = -2.26$, $P < 0.05$) (Figure 2).

To avoid spurious relationships, we first checked if the vegetation variables were independent (Table 1 reports variable summary). We found no correlation ($P > 0.05$) between (1) plant morphospecies richness and number of trees with dbh $\geq$ 5 cm (Spearman, $r = -0.15$, ns), (2) morphospecies and *Alluaudia procera* abundance (Spearman, $r = -0.09$, ns), and (3) number of trees with dbh $\geq$ 5 cm and *Alluaudia procera* abundance (Spearman, $r = 0.79$, ns). We found that sifaka abundance (number of individuals found in each forest fragment) correlated with the proportion of trees with dbh $\geq$ 5 cm (Spearman, $r = 0.97$, $P < 0.013$) but not with forest fragment area (Spearman, $r = 0.42$, ns) or plant morphospecies richness (Spearman, $r = -0.15$, ns). *Alluaudia procera* abundance (range: 42–107 plants; mean ± SE: 65.7 ± 32.1 plants) correlated with sifaka abundance (Spearman, $r = 0.90$, $P < 0.05$) but not when Bonferroni correction was applied.

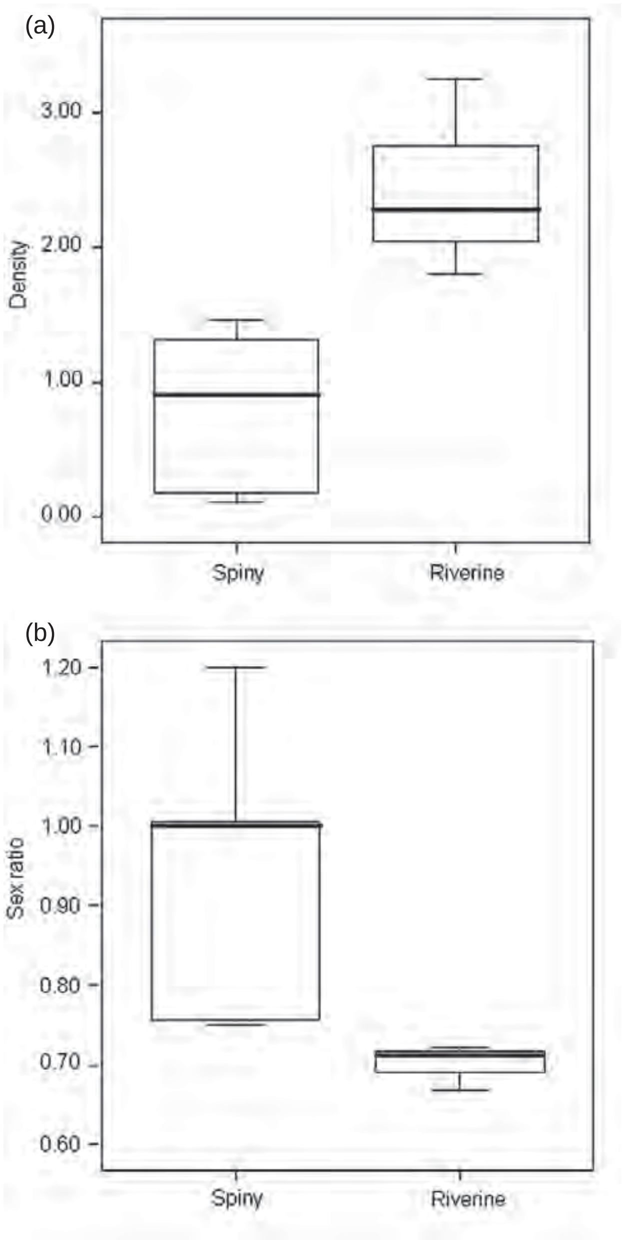

Figure 2. Box plot showing the difference between the spiny fragments (n = 6) and riverine forest areas (n = 3) for sifaka density (ind. ha^{-1}) (a) and sex ratio (b). Density values relate to 183 adult individuals in total; sex ratio for 102 adult males and 76 adult females. All differences are significant. Solid horizontal lines: medians; box length: interquartile range; thin horizontal lines: observed value range.

The Berenty Estate mostly hosts small (1–10 ha) to medium-sized (10–100 ha) fragments (*sensu* Marsh 2003) all created with the foundation of a 5000-ha sisal plantation and the establishment of protected sites in 1936, in agreement with Tandroy clans. Since fragmentation is not at its early stages, we did not expect a random sifaka distribution across fragments. A major predictor of sifaka distribution is probably river water availability because sifaka density decreases as the distance from the river increases and it is higher in the riverine forests (Ankoba and Malaza; 1.8–3.25 ind ha^{-1}) than in the spiny forest fragments (0.09–1.31 ind ha^{-1}) (Figure 2).

Other than density, fragmentation can be related to male-skewed sex ratio (Ramanamanjato & Ganzhorn 2001). Consistently, we found a generally higher percentage of males across forest fragments. However, sex ratio was weakly biased in the spiny forest fragments, possibly due to sifaka habituation to harsh but steady conditions, and strongly male skewed in Berenty riverine forests, as the possible result of the decreased availability of lemur staple food (provided by tamarinds) and the increased competition over food by the introduced *Eulemur fulvus* Grandidier 1871) × *E. collaris* (Geoffroy, 1812) (Norscia & Palagi 2008; Figure 2).

Within spiny forest fragments the sifaka situation is puzzling, with density varying from low (0.09 and 0.27 ind ha^{-1} at Anjapolo and West Rapily, respectively) to high values (0.58–1.31 ind ha^{-1} for the other fragments), almost spanning minimum and maximum densities recorded for other sifaka species in other dry deciduous forest fragments of Madagascar (from 0.03 to 0.90–1.73 ind ha^{-1}; Müller *et al.* 2000, Quéméré *et al.* 2009). While low densities in fragments may be a consequence of habitat contraction, high densities can result from different factors, such as home-range contraction and edge and refugium effects. Home-range shrinkage (as observed for *P. diadema*; Irwin 2008) may be a possibility for Fragment X, whose area is at the lower limit of *P. verreauxi* home range (3 to > 8 ha depending on sites; Norscia & Palagi 2008) but it is unlikely for larger fragments. Positive edge effect (Ganzhorn 1995) cannot be considered because the vegetation is overexposed to sunlight both at the boundary and inside fragments. Instead, we suggest that forest fragments have a refugium value (as for *Avahi meridionalis*; Norscia 2008). Considering that sifaka are able to move through and partially exploit the surrounding matrix, a likely possibility is that sifaka leak out from degraded or overpopulated forest fragments (such as sifaka-packed Ankoba and scrub areas: Norscia & Palagi 2008; or the Tandroy spiny forest of Bedaro, exploited and inhabited by people) and use the protected spiny forest fragments as a shelter zone. The refugium hypothesis, which also predicts density values possibly inflated by small area, is consistent with the lack of correlation between sifaka abundance and fragment area.

Sifaka abundance correlated, instead, with the proportion of large trees (dbh $\geq$ 5 cm) and showed a correlation trend with *Alluaudia procera* abundance, probably because (1) large trees are an important degradation indicator, can approximate relevant vegetation, and are particularly important for vertical leapers (Norscia 2008, Ramanamanjato & Ganzhorn 2001); (2) low values of dbh, reflecting low leaf availability, negatively affect folivores (Ganzhorn 1995); (3) *Alluaudia procera* is both an important food species for the sifaka and a crucial component of the spiny forest vegetation (Elmqvist *et al.* 2007, Wehr 2010).

Our results suggest that intrinsic factors (density and sex-ratio) do not shed light on population viability (due to their heavy fluctuation) and that, among extrinsic factors, vegetation structure overrides fragment size in dictating animal distribution in the small spiny-forest fragments. This scenario is expected in case of patchy animal populations using small patches as refugia, surrounded by an inhospitable matrix and with no source of continuous habitat to rely on. However, fragmentation per se is a landscape-scale process and a landscape-scale study on many species is needed to draw final conclusions in this respect.

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Sexual Signalling in *Propithecus verreauxi:* Male "Chest Badge" and Female Mate Choice

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Abstract

Communication, an essential prerequisite for sociality, involves the transmission of signals. A signal can be defined as any action or trait produced by one animal, the sender, that produces a change in the behaviour of another animal, the receiver. Secondary sexual signals are often used for mate choice because they may inform on a potential partner's quality. Verreaux's sifaka (*Propithecus verreauxi*) is characterized by the presence of two different morphs of males (bimorphism), which can show either a stained or clean chest. The chest becomes stained by secretions of the sternal gland during throat marking (rubbing throat and chest on a vertical substrate while smearing the scent deposition). The role of the chest staining in guiding female mate choice was previously hypothesized but never demonstrated probably due to the difficulty of observing sifaka copulations in the wild. Here we report that stained-chested males had a higher throat marking activity than clean-chested males during the mating season, but not during the birth season. We found that females copulated more frequently with stained-chested males than the clean-chested males. Finally, in agreement with the biological market theory, we found that clean-chested males, with a lower scent-releasing potential, offered more grooming to females. This "grooming for sex" tactic was not completely unsuccessful; in fact, half of the clean-chested males copulated with females, even though at low frequency. In conclusion, the chest stain, possibly correlated with different cues targeted by females, could be one of the parameters which help females in selecting mates.

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Introduction

Communication, an essential prerequisite for sociality, involves the transmission of signals [1,2]. A signal can be defined as any action or trait produced by one animal, the sender, that produces a change in the behaviour of another animal, the receiver [3]. The transfer of messages, either born or not by signals [4], can be beneficial to either senders, receivers, or both [5]. Secondary sexual signals (visual, acoustic or chemical) are often used for mate choice because they may inform on a potential partner's quality [6]. In bipedal vertebrates, mate choice often relies on visual sexual signals that are placed frontally to the observer. This situation occurs quite frequently in birds. Peacock (*Pavo cristatus*) tail spreading [7], the level of symmetry in chest plumage of male zebra finches (*Taeniopygia guttata*) [8], and the size of the black feather bib on the throat of male house sparrows (*Passer domesticus*) [9] are just three examples of secondary visual sexual signals used by females to choose mating partners. Within the primate order, some observers have reported that also in humans (*Homo sapiens*), women's sexual selection appears to be influenced by the amount of chest hairs in males [10]. Men's choice can be affected by size and symmetry of women's breasts [11–13], a signal that in humans is exaggerated compared to other primates [14,15]. Quadruped locomotion habits and chest sexual signals do not generally co-exist due to obvious perceptual constraints.

Frontal visual signals can be favoured by sexual selection when three conditions are met: 1) a diurnal lifestyle, which makes visual signals detectable; 2) upright locomotion, which makes face and/or chest signals visible; 3) a mating system based on either female or male mate choice and strong intra-sexual competition [16].

In primates, besides humans, only a few species meet such conditions [17]. Orang-utans are one of the most sexually dimorphic apes with dimorphism in size, adornments, and vocal signals [17]. Orang-utans are characterized by an irreversible bimorphism and fully mature males can show frontal sexual adornments, which consist of cheek flanges and a throat pouch, a sort of chest "badge" [18]. Males without such secondary sexual features are generally named as "unflanged" males which, under particular social circumstances (e.g. the absence of a flanged male), can acquire in a few months the adornments typical of flanged males [19].

Verreaux's sifaka (*Propithecus verreauxi*) is a diurnal prosimian species characterized by upright locomotion (bipedal hopping and leaping; [20]) and male intra-sexual competition [21]. There is a lack of sexual dimorphism in body size and females are dominant over males [20,22]. These characteristics make it impossible for males to coerce female copulation and promotes female mate choice [20,22–24]. Lewis [25] reported bimorphism in male sifakas, which can show either a stained or unmarked chest, as a function of scent-marking activity during which the throat and chest are rubbed up against a substrate, often multiple times within a single marking bout [26]; Table 1. (Fig. 1). In prosimians, glandular scent-marking has a variety of social functions such as advertisement and territorial defense (*Propithecus verreauxi*, [25]; *Propithecus edwardsi*, [27]; *Lemur catta* [28]), intergroup communication (*Propithecus verreauxi* [25]), advertisement of social dominance (*Lemur catta*, [29]), signaling of reproductive condition (*Lemur catta*, [30,31]), and mate selection (*Nycticebus pygmaeus* [32]; *Propithecus verreauxi*, [22]). About half of the scent marks by sifaka males are overmarks, in which a scent mark is placed on or near a female scent mark [25] and thus, in cases of intense activity, the staining of the chest is probably a combination of a male's own glandular secretions, female anogenital secretions, female urine, and dirt [26]. Lewis and van Schaik [26] described this male phenotypic variation (stained versus clean-chested males) as a form of reversible bimorphism. However, the authors did not find any clear evidence that the two morphs of males differ in their intrinsic physical characteristics, such as body size and maxillary canine length [26]. Norscia et al. [22] demonstrated that females gave copulatory priority to males who more frequently countermarked female scent depositions. However, results demonstrating a clear link between male chest bimorphism and female mate choice are still lacking.

Here, we decided to test whether or not sifaka females' mating patterns are associated with the male chest badge, which seems to correlate with male scent-marking and dominance [25,26]. We made the following predictions:

Prediction 1

Lewis's findings [25] suggest that while clean-chested males deposit scents for inter-group communication, stained-chested males release scent depositions for mate-guarding purposes. Moreover, during the birth season, testes mass (and, possibly, in testosterone levels) do not differ between clean- and stained-chested males [33]. If the stained chest is a signal linked to male intra-sexual competition (ultimate cause) and to testosterone levels (proximate cause), we expect stained-chested males to show a higher throat-marking frequency than the clean-chested males during the mating season but not necessarily during the birth season.

Prediction 2

Lewis and van Schaik [26] reported that stained-chested males are generally dominant in their social groups. Thus, if a stained chest is one of the possible signals which females can use in their mate choice, we predict stained-chested males will copulate more frequently than the clean-chested males.

Prediction 3

In the mating market, the balance of power tilts in favor of females whenever males cannot force them into mating [24], especially when females are dominant. Consequently, males depend on females for breeding opportunities and must compete to prove their superiority to females, thus increasing their possibility to be selected [24,34]. Males can engage in both contest competition via physical/ritualized fighting and outbidding competition, in which a male plays off rivals by making a better offer [35]. In the latter case, males can secure the favors of a female by advertising their quality (e.g. the dominance status) through visual or olfactory displays [36,37] and/or by providing commodities in exchange for female access [38,39]. In sifaka, the mating system follows the biological market rules where both scent-marking and grooming are good male services on which females base their mate selection [22]. Norscia et al. [22] found that to obtain priority and/or a high number of copulations sifaka males had to be top-scent releasers and/or females' top-groomers. According to the biological market theory, we expect that clean-chested males, with lower scent-releasing potential [26], in order to have some copulation opportunities need to compensate by offering more grooming to females than stained-chested males.

Results

Prediction 1 supported

During the mating season stained-chested males (mean ±SE: 2.07±1.15 times per hour) throat marked significantly more often than clean-chested males (mean ±SE: 0.19±0.11 times per hour) (two independent samples randomization test: $t = -1.789$; $n_c = 6$, $n_s = 5$, $p = 0.018$; Fig. 2). A significant difference was also found in the use of genital glands by the two morphs of males (stained-chested males, mean ±SE: 0.29±0.17 times per hour; clean-chested ones, mean ±SE: 0.02±0.01 times per hour; two independent samples randomization test: $t = -1.688$, $n_c = 6$, $n_s = 5$, $p = 0.045$).

During the birth season the difference in the throat-marking between stained- and clean-chested males disappeared (stained-chested males, mean ±SE: 3.52±1.03 times per hour; clean-chested ones, mean ±SE: 0.54±0.26 times per hour; two independent samples randomization test: $t = 2.801$, $n_c = 3$, $n_s = 3$, $p = 0.140$); no difference was also found for genital depositions (stained-chested males, mean ±SE: 1.49±0.19 times per hour; clean-chested ones, mean ±SE: 0.07±0.18 times per hour; two independent samples randomization test: $t = 7.151$, $n_c = 3$, $n_s = 3$, $p = 0.105$). The seasonal difference in the sample size (11 males, mating season; 6 males, birth season) is due to the presence of out-group males in our study groups during the mating period [40].

Table 1. Description of the behaviours recorded during the study.

Behavioural items	Description
Mating event	Copulatory behaviour in which intromission and thrusting are unambiguously observed. Ejaculation, generally not visible, is inferred based on a rapid increase in thrusts and a pause just prior to the dismount, followed by intense genital self-grooming [45,55].
Grooming	Fur-cleaning, which in strepsirhines is typically performed via tooth-comb.
Genital marking	The genitals are rubbed on the substrate and scent deposition is released. Both males and females perform genital marking.
Throat marking	Animals rub their throat and chest on a vertical substrate in a repeated manner while smearing the scent deposition. Throat marking is a dimorphic behaviour, in fact only males perform it.

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Figure 1. The two different morphs of sifaka males. An example of stained-chested male (on the left, photo by E. Palagi). The brown smear, particularly evident on the throat, extends to the upper part of the chest (dark/brown, photo by I. Norscia). An example of clean-chested male (on the right). No brown smear is present on the throat.
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Prediction 2 supported

In the mating season, the stained-chested males engaged in significantly more copulation events per hour (mean ±SE: 0.59±0.12) than the clean-chested ones (mean ±SE: 0.12±0.06) (two independent samples randomization test: $t = -3.587$, $n_c = 6$, $n_s = 5$, $p = 0.0016$; Fig. 3).

Prediction 3 supported

In the mating season females received more grooming from clean-chested males (mean ±SE: 0.06±0.02 times per minute) than from stained-chested ones (mean ±SE: 0.14±0.00 times per minute) (paired samples randomization test: $t = 2.035$, $n = 6$, $p = 0.028$; Fig. 4a). This difference vanished in the birth season (paired samples randomization test: $t = -0.81$, $n = 6$, $p = 0.499$; Fig. 4b).

Discussion

In this paper, we found that stained-chested males had a higher throat and genital-marking activity than clean-chested males during the mating season but not during the birth season (Prediction 1 supported). Moreover, we found that females copulated more frequently with stained-chested males (including both resident and non-resident) than with clean-chested males (including both resident and non-resident) (Prediction 2 supported), even though the latter offered more grooming to females compared to the former during the mating season (Prediction 3 supported).

Males of several mammalian species modulate their scent-marking activity according to their perceived mating opportunities [41,42] and can increase their plasma testosterone concentration, as well as scent-marking, when they are sexually stimulated [43]. In the mating season a scent-marking dichotomy between the two different morphs of sifaka males existed (stained-chested males scent-marked more frequently than clean-chested ones). This

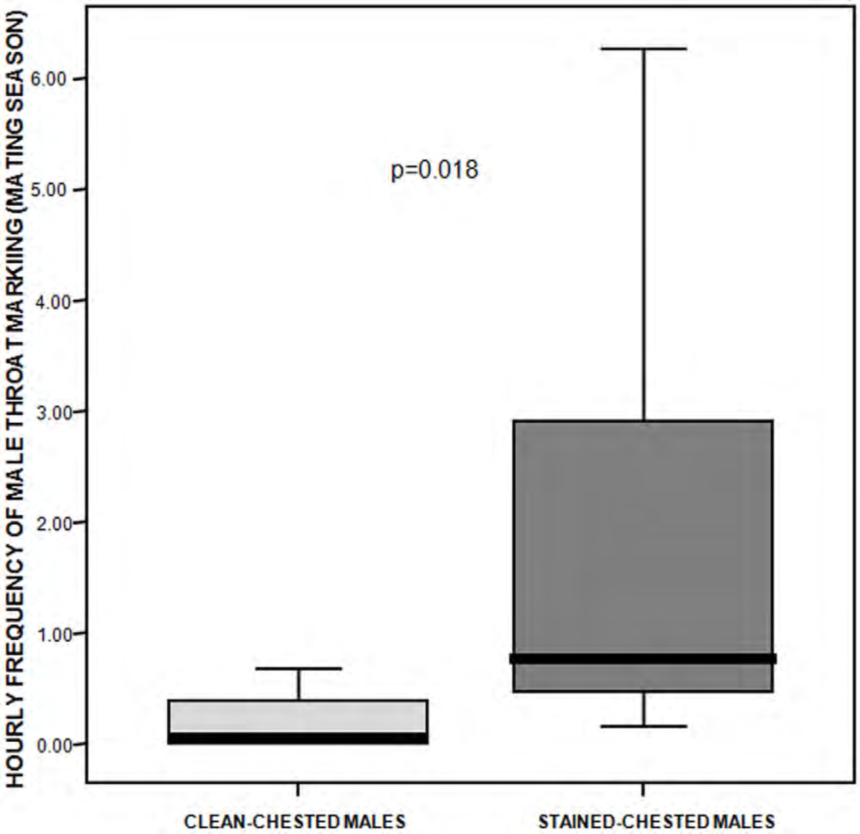

Figure 2. Marking activity in the mating season. Frequency of throat marking performed by clean- and stained-chested males during the mating season. Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values.
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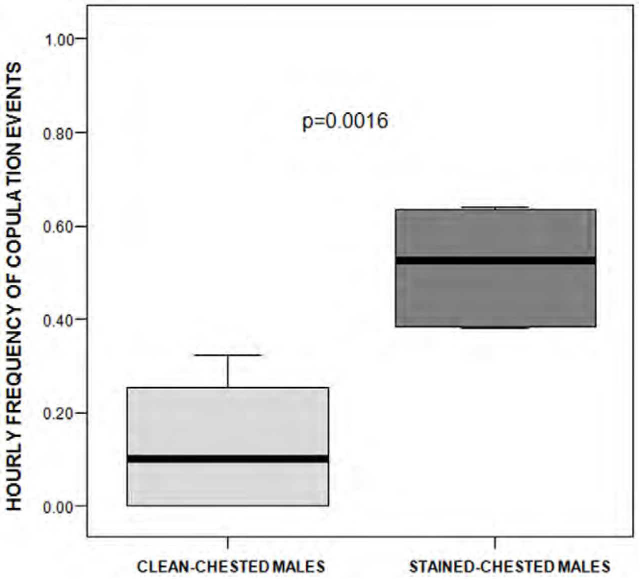

Figure 3. Copulation events of the two different morphs of males. Frequency of copulation events performed by clean- and stained-chested males. Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values.
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dichotomy disappeared during the birth season, when males were not sexually stimulated and males' intra-sexual competition decreased due to the lack of eggs to be fertilized. One of the proximate causes of the scent-marking dichotomy in the mating season is the difference in the concentration of testosterone levels between stained- and clean-chested males, which also differ in their testes mass [33]. The lack of difference in the testes mass of the two male morphs during the birth season led authors [33] to infer that stained- and clean-chested males do not differ in their testosterone levels. This is consistent with our data, which show no difference in the frequency of scent-marking rates between the two morphs of males in the birth season.

The stained-chest provides benefits to sifaka males by increasing their reproductive opportunities ("marking for sex" tactic). Copulations involved both in-group and out-group stained-chested males, this suggests that the chest badge can be functional to females, especially when they have to gather information on less familiar out-group males. This interpretation is supported by another recent finding obtained from Beza Mahafaly sifaka population, where it has been observed that most (29 of 52) of males sired at least one offspring outside their resident group [44].

Lewis and van Schaik [26] underlined the importance of multimodal signaling in *Propithecus verreauxi*, in which the additional visual cue of a chest stain enhances the information transmitted via the olfactory signal produced by the scent glands. Signals are frequently made up of multiple components that interact with each other to alter the receiver's response [4,44–46]. Such multiple signals were defined as multimodal (composed of signals related to different sensory modalities) [47,48]. The multimodality of sifaka communication is linked to its diurnal habits [20]. In fact, diurnal prosimians use multimodal signals in both reproductive and nonreproductive contexts [26,49,50]. Two different studies showed that both *Propithecus edwardsi* and *Microcebus murinus* females use multimodal estrus advertisement by associating a particular vaginal morphology with vocalizations [51,52]. Palagi et al. [49] and Palagi and Dapporto [53] described urine-marking in *Lemur*

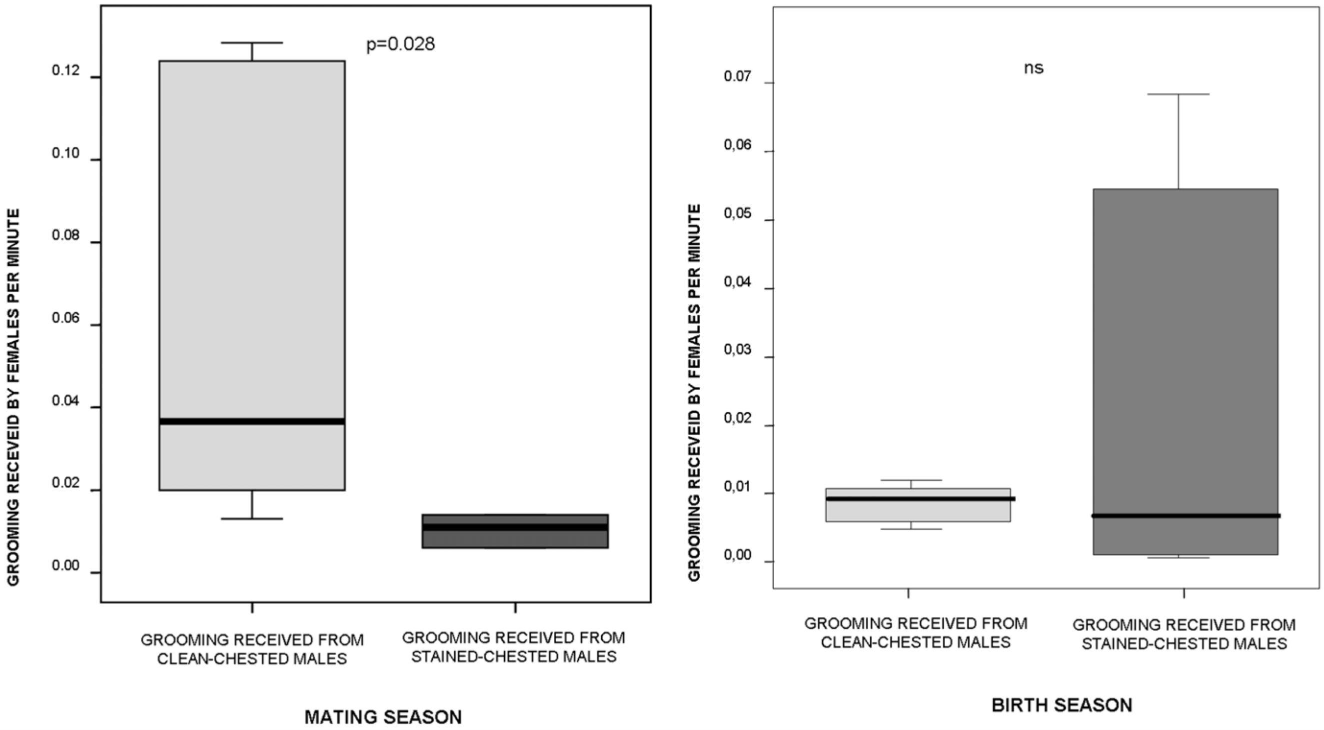

Figure 4. Grooming received by females. Frequency of grooming received by each female from clean- and stained-chested males (a) during the mating season and (b) during the birth season. Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values.
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catta as a multimodal signal composed by an olfactory cue (urine) and a visual cue (tail up, increasing the detection probability). Switching from unimodal (one cue) to multimodal signalling (more than one cue) may increase the probability of sifaka males to be promptly detected by females. Sifaka males adjust the intensity of their signal by varying its delivery frequency. Maintaining the visual chest badge is likely to be costly because it requires much effort in renewing scent depositions.

In many non-human primate species, grooming is a commodity which can be exchanged for itself or for breeding opportunities [54–56]. Grooming is one of the behaviors most frequently involved in the biological market system [35]. Within a mating marketplace, low quality males are expected to overcompensate for their quality by providing more grooming to oestrous females. Similarly, a male of high quality may be preferred by the females, and will pay a lower grooming price to be favored by them. This prediction has been supported by data coming from chimpanzees. In this species, low-ranking males need to provide more grooming to oestrus females than high-ranking males in order to gain access to females [56]. As in other primate species, grooming also seems to play an important role in sifaka. Norscia et al. [22] found that in the months immediately preceding the mating season, male grooming of females positively correlated with female grooming of males. In the mating period, this correlation disappeared because grooming was exchanged by males for copulations (''grooming for sex'' tactic). Therefore, it is not surprising that during the mating season clean-chested males, due to their low testosterone levels and consequent low production of secretions (this paper; [33]), invest much more in the ''grooming for sex'' tactic with females than stained-chested males do. In contrast, the birth season was characterized by a lack of difference in the grooming received by females from the two morphs of males. The ''grooming for sex'' tactic adopted by clean-chested males during the mating season is not completely unsuccessful; in fact, half of the clean-chested males under study did copulate with females, even though their copulation frequency was significantly lower than that of stained-chested males (Figure 2). The observation that copulation frequency is higher in stained-chested males (usually dominant in their social group; [26]) than in clean-chested males is consistent with the paternity test results presented by Kappeler and Schäffler's [57], showing that sifaka dominant males can sire up to 90% of infants.

In conclusion, since the badge depends on testosterone, scent-marking, and dominance, it can represent an ''overview'' of males' physical state. To demonstrate the function of a potential communicative signal the experimental approach is generally required, unfortunately such approach is not feasible with this species.

Our findings that females copulate more with males showing chest stain suggest that this cue is used by females to choose mates. The choice pattern could also result from correlated expression of the stained chest with other cues that the females directly target. The clues conveyed by the badge may be used as an additional piece of information to assess the potential quality of stranger males, possessing cues that cannot be timely accessed by females.

The presence of the multiple mating tactics, ''marking for sex'' (stained-chested males) and ''grooming for sex'' (an alternative, but not completely functional, tactic used by clean-chested males) may be a means by which sifaka population buffers the inbreeding phenomenon in the small, isolated fragment of the Berenty forest [58].

Materials and Methods

Ethics statement

This study was approved by University of Pisa (Animal Care and Use board). Since the study was purely observational the committee waived the need for a permit. The study was conducted with no manipulation of animals. The study was carried out in the private Reserve of Berenty (South Madagascar) and De Heaulme family (the owner) permitted us to observe animals.

Study species and site

We conducted this study in the secondary forest of Ankoba, part of the 140-ha Berenty forest fragment (South Madagascar; S 24.99°; E 46.29°; for an extensive description see [59] on *Propithecus verreauxi* (Verreaux' sifaka). At Berenty, sifaka groups range from 1 to 10 individuals, according to a complete census conducted in November-December 2006 [60]. They inhabit riverine and dry forests of south and southwest Madagascar [61]. Females usually experience a single oestrus period (2–3 days) per year and both sexes can mate with multiple partners in their own and neighbouring groups, especially when a single group offers suboptimal mating opportunities [52]. In particular, males can start roaming and visiting other groups in search of oestrus females [21]. The short oestrus period and the fact that mating can be tightly synchronized within a population make copulations very difficult to detect and observe [23,26]. Moreover, at Berenty, cyclones and heavy raining followed by river flooding normally prevent data collection in the period January-February, coinciding with sifaka's mating period. In 2007, for the first time it was possible to gather data on mating because of a prolonged drought involving South Madagascar. In the end, we gathered the highest sample of mating episodes (57 copulations) ever recorded in sifaka [22]. In May-July 2008, during the birth season, we gathered data on the same groups. This additional sample collection permitted us to compare data on marking behaviour and male-female grooming between the two different seasons (mating 2007-birth 2008).

Observational data and operational definitions

The study was conducted on adults of two sifaka groups in two different periods (mating season: 11 adult males, 6 adult females; birth season: 6 adult males, 6 adult females). Within the out-group males observed in the mating period, 2 were stained-chested and 3 were clean-chested. Animals were followed from dawn to dusk by *focal* (collection of grooming data) (mating season: 501 hours, birth season: 368 hours) and *all occurrences animal sampling* (collection of olfactory activity and copulation data) (mating season: 221 hours, birth season: 258 hours). During the mating season the authors and a field assistant collected data with daily observations of about 11 h/day. During the birth season, due to the reduced day length, the observations decreased to about 9 h/day. As typical of the sifaka the individuals of the group usually moved, rested, and foraged cohesively. However, the group could split during the mating days: in this case, the observers separated to follow the two different subgroups. We individually identified the animals according to their external features (scars, fur patches, fur color, [62]).

To distinguish stained- and clean-chested males we used the descriptions given by Lewis and van Schaik [26]. We photographed males' chest at a maximum distance of 2 m. Males with a brown, greasy spot on the chest were labeled as ''stained'', whereas males with a white, clean chest were identified as ''clean''. The animals with intermediate color were two out-group males (one per group) which spent in our study groups only few hours in a day. For this reason we decided to exclude them from the analysis.

Brockman [21], who observed sifaka mating in a different study site (Beza-Mahafaly; Southeastern Madagascar), provided the operational definitions used during this study (Table 1). We included in the analyses only proper copulations.

Statistical analyses

Due to the small sample size and deviation from normality (Kolmogorov-Smirnov<0.05) we used randomization procedures ([63], software: Resampling Procedures 1.3 by David C. Howell, freeware). Specifically, randomization tests were employed with a number of 10,000 permutations using resampling procedures. The software provides a t value in the same way as in a standard t test, but calculates a p value as the proportion of randomized datasets that yield an even more extreme outcome. The analyses were conducted at an individual level. All analyses were two-tailed, and the level of significance was set at 5%.

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Author Contributions

Conceived and designed the experiments: IN EP. Performed the experiments: SD IN DA EP. Analyzed the data: SD IN DA EP. Contributed reagents/materials/analysis tools: EP. Wrote the paper: SD IN EP.

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Signalisation sexuelle dans le lémurien *Propithecus verreauxi*: le badge sur la poitrine des mâles et le choix de partenaires sexuels par les femelles

Introduction

La communication, une condition essentielle pour la socialité, implique la transmission de signaux. Un signal peut être défini comme une action ou une caractéristique produite par un animal, l'émetteur, qui produit un changement dans le comportement d'un autre animal, le récepteur. Des signaux sexuels secondaires sont souvent utilisés pour le choix du partenaire, car ils peuvent informer sur la qualité d'un partenaire potentiel.

Le sifaka de Verreaux (*Propithecus verreauxi*) est caractérisée par la présence de deux formes différentes de mâles (un phénomène dénommé bimorphisme), qui peuvent montrer soit une poitrine tachées ou propre. La poitrine est taché par des sécrétions de la glande sternale pendant le marquage effectué avec la gorge (à travers le frottement de la gorge et de la poitrine sur un substrat vertical qui permit de bien étaler l’odeur). Le rôle de la coloration de la poitrine pour guider le choix du partenaire féminin a été précédemment supposé, mais jamais démontré probablement due à la difficulté d'observer des copulations de sifaka.

Résultats et Discussion

Nous rapportons ici que les mâles avec la poitrine tachée avaient une activité supérieure de marquage à travers la gorge que les mâles avec la poitrine propre pendant la saison des amours, mais pas pendant la saison de naissance. Nous avons constaté que les femelles ont copulé plus souvent avec les mâles tachés que avec les mâles propres. Enfin, en accord avec la théorie du marché biologique, nous avons constaté que les mâles avec la poitrine propre, offraient plus de toilettage aux femelles que les autres mâles probablement pour compenser leur capacité inférieur de relâcher leur odeur. Cette tactique de "toilettage pour le sexe" était pas complètement échoué. En fait, la moitié des mâles avec la poitrine propre a réussi à copuler avec des femelles, même si à basse fréquence. En conclusion, le badge sur la poitrine peut être un des paramètres qui aident les femmes dans le choix de partenaires sexuels.

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Se gratter autour de l'accouplement: les facteurs qui affectent l'anxiété chez le lémurien *Lemur catta* en liberté

Introduction

Le comportement de grattage (ou auto-grattage, *self-scratchin*g) a été utilisé avec succès chez les primates (des lémuriens à l'*Homo sapiens*) pour détecter le niveau d'anxiété, qui normalement corrèle avec le niveau de stress. Ici, nous avons étudié la fluctuation du comportement du grattage en *Lemur catta* pendant la saison des amours, dans la forêt de Berenty (Sud Madagascar). En particulier nous avons évalué si le grattage (1) variait selon le sexe et les différences de rang, (2) augmentait pendant la période de stress maximum (autour des jours d'accouplement) et (3) se réduisait après un session de toilettage (*grooming*).

Nous avons suivi deux groupes de *Lemur catta* (23 invidus adultes/subadultes) et nous avons collectés des données sur les comportement d'auto-grattage (*self-scratching*), agression, et toilettage. Basé sur les caractéristiques de la région du périnée, qui se gonfle et change de couleur dans les jours fertiles, nous avons reconnu deux périodes: période de gonflement faible (LS), sans femelles en œstrus, et période de gonflement élevé (HS), lorsque au moins une femme était en œstrus.

Résultats et Discussion

Notre première prédiction était que les comportements agressifs et le grattage lié aux niveaux d'anxiété pouvait covarier. En effet, les fréquences de grattage étaient maximales dans la périod HS, quand les niveaux d'agression étaient aussi les plus élevés. En accord avec la littérature précédente, ce résultat suggère que les conflits autour de jours d'œstrus peuvent augmenter les niveaux d'anxiété dans le groupe social.

Nous nous attendons aussi que les niveaux de grattage étaient plus élevés chez les mâles, parce qu'ils compétent agressivement pour les femelles, qui choisissent les partenaires sexuels et effectuent des attaques répétées sur le mâles. Contrairement à notre prédiction, les taux de grattage étaient similaires chez les mâles et les femelles, probablement parce que la forte concurrence, qui implique les deux sexes, tend à éliminer les différences intersexuels. Les comportement de grattage ne dépend pas du rang et ce résultat contraste aussi avec notre prédiction d'une influence du range sur le niveau d'anxiété. Ce résultat probablement dépend du fait que la hiérarchie des animaux changeait de la période LS à la période HS. Enfin, nous avons montré que chez les *Lemur catta*, ainsi que dans d'autres primates, le comportement grattage diminuait après des sessions de toilettage réciproque dans les deux périodes. Ce dernier résultat suggère que le toilettage peut aider à réduire l'anxiété dans les strepsirrhiniens.

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ORIGINAL ARTICLE

Scratching around mating: factors affecting anxiety in wild *Lemur catta*

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Abstract Scratching has been successfully used to detect anxiety, a proxy for stress, in primates, from strepsirrhines to *Homo sapiens*. Here, we investigated the fluctuation of scratching in *Lemur catta* during the mating season. In particular we evaluated whether scratching (1) varied according to sex and rank differences, (2) increased in the period of maximum stress (around the mating days), and (3) was reduced by grooming. At Berenty (South Madagascar), we followed two lemur groups (23 adult/subadult individuals) and gathered data on self-scratching, aggression, and grooming. Based on perineal area features, we recognized two periods: low swelling (LS), with no estrus female, and high swelling (HS), when at least one female was in estrus. We predicted that aggressive behaviors and anxiety-related scratching would covary. Indeed, scratching peaked in HS, when aggression was also highest. In agreement with previous literature, this result suggests that conflicts around estrus days may raise anxiety levels in the social group. We expected scratching levels to be highest in males because they aggressively compete for females and are subject to mate choice and repeated attacks by dominant females. Instead, the scratching rates were similar in males and females, probably because the high competition, which involves both sexes, dampened intersexual differences. In contrast to our prediction, scratching was not rank dependent, probably because animal ranking positions changed from LS to HS. Finally, we showed that, in ring-tailed lemurs, as well as in other primates, scratching decreases after reciprocal grooming in both periods. This finding provides the first evidence that grooming could assist in reducing anxiety in strepsirrhines.

Keywords Aggression · Grooming · Estrus · Ring-tailed lemurs · Sex · Human and nonhuman primate stress

Introduction

In *Homo sapiens*, nonhuman primates, and other mammals, anxiety, a proxy for stress, is an emotional state involving tension and/or agitation, with both physiological and behavioral implications (Craig et al. 1995; Van Riezen and Segal 1988; Barros and Tomaz 2002; Bourin et al. 2007). There is evidence that self-directed behaviors, including self-scratching, self-grooming, yawning, and body shaking, can provide an index of anxiety (Maestripieri et al. 1992; Schino et al. 1996; Aureli et al. 2002), even if not necessarily of general stress levels (Troisi 2002; Higham et al. 2009). Self-scratching (hereafter scratching), in particular, appears to be one of the most reliable behavioral tools to measure anxiety. Indeed, anxiety states can share common biochemical origins with the physiological sensation of pruritus (*sensu* Rothman 1941), leading to the itch–scratch cycle (Shankly 1988; Stangier et al. 2003; Tran et al. 2010). In *Homo sapiens*, anxiolytic substances (e.g., nitrazepam and diazepam) reduce pruritus and scratching (Krause and Shuster 1983; van Moffaert 2003). Schino et al. (1991) found that in

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female long-tailed macaques (*Macaca fascicularis*) scratching rates decreased after pharmacological manipulation of mood through anxiolytic (lorazepam). Maestripieri et al. (1992) observed that acute administration of the anxiolytic midazolam reduced the rate of scratching in infant rhesus monkeys (*Macaca mulatta*). Similarly, in common marmosets (*Callithrix jacchus*) and black-tufted marmosets (*Callithrix penicillata*), administration of the anxiolytic diazepam induced a significant reduction in scratching (Cilia and Piper 1997; Barros et al. 2000). In mice with genetic deletion of Sapap3 that exhibit increased anxiety, rough self-grooming—a form of scratching leading to facial hair loss—increases. This scratching was alleviated by a selective serotonin reuptake inhibitor (Welch et al. 2007).

In primates, there is strong behavioral evidence that scratching increases in anxiogenic situations. In *Homo sapiens*, for example, tense situations increase rates of body scratching (Morris 1977; Fried 1994; Troisi et al. 2000; Tran et al. 2010). In chimpanzees, *Pan troglodytes*, scratching levels were particularly high in crowding conditions (Aureli and de Waal 1997) as well as among females and subordinate males when a nonaffiliative group member was in their proximity (Kutsukake 2003). In cercopithecines, self-directed behaviors, including self-scratching, have been found to increase in the presence of potentially threatening dominant neighbors (olive baboons, *Papio anubis*: Castles et al. 1999). The only two studies available for strepsirrhines indicate that scratching increases after predatory attacks (Palagi and Norscia 2011) and during intergroup encounters (Nunn and Deaner 2004).

Based on the previous framework, it is clear that scratching fluctuations can provide an index of anxiety. In the present study, we investigated the factors affecting anxiety levels, measured via scratching rates, during the mating period in *Lemur catta*, a Malagasy social strepsirrhine. In particular, we tested the following predictions:

Prediction 1: scratching and rank

In species with despotic hierarchies and in which the ranks can rapidly shift over time, high-ranking individuals have the greatest physiological indices of anxiety, because they must frequently reassert their domination over subordinates (Sapolsky 2005). *Lemur catta* is highly despotic (Jolly 1966; Kappeler 1993), and the rank position of dominants can change during the mating period (Jolly 1966). Indeed, dominants show the highest stress levels measured via fecal cortisol (Cavigelli 1999). Since anxiety is a subset of stress (Higham et al. 2009), we expect scratching levels to be highest in high-ranking individuals (prediction 1).

Prediction 2: scratching and sex differences

Lemurs are seasonal breeders (Fleagle 1999). Females are dominant over males, and females actively perform mate choice (Jolly 1966; Koyama 1988; Norscia et al. 2009). In *Lemur catta* females normally experience a single estrus per year, lasting a few hours (Evans and Goy 1968; Koyama 1988; Cavigelli and Pereira 2000). Males incessantly chase other males before being able to mount the female and are aggressed, and often wounded, by females during mating attempts (Jolly 1966; Sauther 1991; Sussman and Richard 1974). In this respect, we predicted that males would experience higher levels of anxiety and, consequently, perform more scratching than females (prediction 2).

Prediction 3: factors modulating scratching

Spanning strepsirrhines to apes, within-group aggressions are one of the main events causing an increase of scratching and other self-directed behaviors in primates (Aureli 1997; Daniel et al. 2008; Fraser et al. 2008; Palagi and Norscia 2011). In *Lemur catta*, within-group aggressions peak during the weeks of female estrus (Jolly 1966). Consequently, we expected scratching to be higher when aggression frequencies peak (prediction 3a).

Primates experiencing anxiety may buffer it by engaging in social interactions (de Waal 1987; Palagi et al. 2004a, 2006; van Wolkenten et al. 2006; Aureli and Yates 2010; Norscia and Palagi 2011). Allogrooming (hereafter grooming) in primates is involved in the establishment and maintenance of social bonds (Dunbar 1988). This social function seems to be mediated by the release of brain opioids such as beta-endorphins (Keverne et al. 1989). Moreover, grooming appears to decrease the heart rate (Aureli et al. 1999; Boccia et al. 1989). Additionally, both giving and receiving grooming can reduce stress-related scratching (Schino et al. 1988; Aureli and Yates 2010). In diurnal lemurs (Barton 1987), including *Lemur catta* (Jolly 1966), grooming is almost always reciprocal. Hence, we expected the scratching by a given individual to be reduced after such individual has engaged in a grooming session, as either groomer or groomee (prediction 3b).

Methods

Study species

Lemur catta (ring-tailed lemur) is a diurnal species showing marked seasonal trends of variation in olfactory behavior, group dispersal, tolerance level, and reproduction (Jolly 1966; Palagi and Norscia 2009; Palagi et al. 2003, 2004b). Females experience an annual estrus of a few days, with receptivity lasting 10–24 h (Jolly 1966; Evans and Goy 1968; Koyama 1988; Cavigelli and Pereira 2000). If they do not get pregnant, a second and third belated estrus are possible (Jolly 1966; Palagi et al. 2003, 2004b). *L. catta* females have a visible estrus, which is asynchronous with other females in their group (Pereira 1991). They experience a genital swelling from about 1.5–3 cm in length and develop a pink center (Jolly 1966). The mating period starts about 1 month before copulations, when the perineal area starts becoming increasingly larger and the center of genitalia increasingly larger and pinker (from small pink to large and bright pink; Jolly 1966). After this preliminary period (low swelling), females experience estrus, specifically referring to the period of high swelling, when vaginal smears are fully cornified and the perineal area is maximally enlarged and pink (Evans and Goy 1968). Generally, receptivity coincides with the last day of maximal pink coloration of vaginal labia (Jolly 1967; Evans and Goy 1968). Thus, color and shape of the genital area are honest signals which reliably indicate imminent ovulation and receptivity (Eaton et al. 1973; Boskof 1978).

Even if no quantitative analyses have been performed so far, many previous reports describe *Lemur catta* hierarchy as linear and highlight the importance of relative ranking position within females, which are dominant over males. A hierarchical change in the alpha female and/or among high-ranking females is a major event for the social group (e.g., Jolly 1966; Kappeler 1990; Palagi et al. 2003; Koyama et al. 2005).

Study site and data collection

This study was performed in the Berenty Forest (South Madagascar, S 25.00°; E 46.30°), characterized by two main climatic periods: a wet season from October to March and a dry season from April to September (Jolly et al. 2006). From mid-March to July 2008, in the period around mating, we observed two groups of *L. catta* (named group A and B). The mating period ended at the end of April for group A and in the first week of June for group B. During the observation period, mean monthly temperatures steadily decreased from 27.93°C (March) to 21.01°C (July) and mean monthly humidity steadily decreased from 68.01% (March) to 63.01% (July). Group A was composed of 6 adult females, 3 adult males, and 1 subadult male, and group B was composed of 6 adult females, 5 adult males, and 2 subadult females. Infants and juveniles were not included in the analyses.

Via 15-min focal sampling (Altmann 1974), we collected 160 h of data (mean ± SE per individual: 16.20 ± 0.33 h) for group A and 229 h (mean ± SE per individual: 17.62 ± 0.24 h) for group B. Aggression was monitored and recorded via all occurrences (Altmann 1974). The animals, habituated to human presence, were sexed and individually identified via facial–body features (Jolly 1966). The observations took place daily from dawn to dusk. Systematic data collection was performed by a total of four observers and was preceded by a training period. Each training period lasted until the interobserver reliability between each dyad of observers ($N = 6$ possible pairs) reached 95% (Martin and Bateson 1986).

We gathered data on grooming, submissive patterns (avoidance and full-spat-grimace, always directed from subordinates to dominant; Pereira and Kappeler 1997), and dyadic agonistic encounters in which we recorded: (1) opponents, (2) conflict type (decided, with a clear winner, or undecided), and (3) agonistic events (chasing, biting, slapping, and aggressive grabbing). We also recorded bouts of scratching, performed using feet and hands, clearly different from self-grooming, which is performed via tooth-comb. Grooming is a behavior state (*sensu* Altmann 1974), and a grooming bout was recorded if grooming lasted at least 10 s; different bouts were recognized when grooming items were separated from one another by at least 10 s. More than 90% of grooming was directly reciprocal, that is, concurrently performed by the two animals or very quickly (<10 s) exchanged by the two animals.

Avoidance, full-spat-grimace vocalizations, and scratching (Pereira and Kappeler 1997) were considered behavior events (*sensu* Altmann 1974), whose very short duration cannot be reliably registered. To avoid recording bias, a single bout of scratching, full-spat-grimace, or aggression was defined as lasting from the first behavioral episode (scratching movement, agonistic interaction, or vocalization) to the last one within a 10-s time slot. We calculated scratching and aggression frequency/minute for each individual.

Operational definitions, data elaboration, and statistical analyses

Only the mating period was considered for the analyses. Within the observation period of each group, we distinguished two subperiods, based on female swelling: the low swelling (LS) period and the high swelling (HS) period. During LS no female was in estrus (*sensu* Evans and Goy 1968) but at least one female showed a change in the perineal area (enlarged size and pink color; Jolly 1966). During HS at least one female was in estrus, thus showing full perineal enlargement and bright pink at the center (Fig. 1), ready to present its genital region as a mating invitation to males (Morris and Bruce 1967). In our study, HS lasted from March 28th to April 29th for group A and from May 13th to June 5th for group B. HS was a continuous period which started with the first female of the group undergoing estrus and ended when the last estrus

Fig. 1 Perineal area of female *Lemur catta* at Berenty, showing no swelling (*left*), low swelling (*middle*), and high swelling (*right*)

experienced in the group was over. LS was the continuous period preceding HS (and defined above). Only LS and HS period were considered in this study.

Hierarchy was determined by applying Matman 1.0 to sociomatrices of dyadic dominance relationships (de Vries et al. 1993). In order to properly assess animal ranking position, a two-step procedure was needed and used. We first verified hierarchy linearity using a test based on Landau's corrected index, h' (step 1). Only after assessing linearity could we use Matman to perform a second analysis: reordering animal ranking positions to fit linearity (step 2). Of course, in case of nonlinear hierarchy, rank fitting via Matman is not reliable. Tests were run on two sociomatrices, one for each period (LS and HS), and both included decided conflicts between group members, avoidance, and full-spat-grimace vocalizations.

We verified the variation of scratching before and after grooming events (sequential analysis). To this purpose, we used the scratching frequencies of each individual recorded during focal observations before the first grooming bout (or in absence of grooming) and after the last grooming bout. The absolute number of scratching bouts was weighted by the minutes either preceding the first grooming bout or following the last grooming bout within the 15-min time slot. The scratching bout/minute rate provided the frequencies per minute.

Due to the small sample size or deviation from normality (Kolmogorov–Smirnov, $p < 0.05$) we applied nonparametric tests (Siegel and Castellan 1988): Wilcoxon's signed-rank test for paired samples, Mann–Whitney's test for independent samples, Spearman's correlation. We selected exact values of probability according to Mundry and Fischer (1998). Analyses were performed via SPSS 12.0 and StatXact 7.0 at the individual level ($N = 23$), with group A and B analyzed independently when rank was involved.

The level of significance (α) was adjusted downward following Bonferroni's technique (Rice 1989). Thus, based on the number of tests run, the α-level was set at 0.01 for tests involving the aggression dataset (α/5) and at 0.0063 for tests involving the scratching dataset (α/8).

Table 1 Changes in ranking position of Berenty ring-tailed lemurs

Group A, LS	Group A, HS	Group B, LS	Group B, HS
T2	TV	MY	MY
MA	MA	CS	CS
TV	T2	CV	SC
MS	MS	SC	BI
T1	T1	BI	CV
BR	BR	CE	BV
BL	BO	BV	CE
BO	BL	2T	2T
RI	GR	CO	PR
GR	RI	PG	PG
		PR	CO
		CI	NE
		NE	CI

Adult females ($n = 6$) are at the top in both groups. Left block: group A hierarchy; right block: group B hierarchy. Both blocks report LS (low swelling) and HS (high swelling) periods

Preliminary analyses

Hierarchy was linear in group A in both LS ($h' = 0.793$, $p < 0.001$) and HS ($h' = 0.982, p < 0.001$) and in group B in both LS ($h' = 0.464$, $p = 0.033$) and HS ($h' = 0.736$, $p < 0.001$). Linearity allowed us to reorder the aggression sociomatrix, thus obtaining the relative ranking position of group members. The hierarchical arrangement in both periods (LS and HS) is presented in Table 1.

Results

In both LS and HS and for both groups we found no correlation (via Spearman's test) between ranking positions

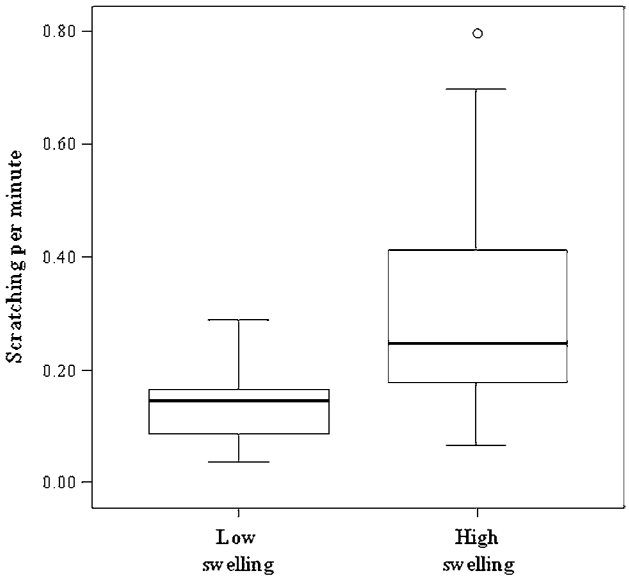

Fig. 2 Difference in the scratching levels (SCR bouts per minute) in the high swelling (HS) and low swelling (LS) period. The difference is significant ($p < 0.001$). *Solid horizontal lines* medians, *box length* interquartile range, *thin horizontal lines* observed value range

and scratching levels: LS (group A: $r_s = 0.375$, $N = 10$, $p = 0.285$; group B: $r_s = -0.081$, $N = 13$, $p = 0.793$) and HS (group A: $r_s = 0.188$, $N = 10$, $p = 0.603$; group B: $r_s = 0.193$, $N = 13$, $p = 0.528$) (prediction 1 not supported).

In the mating period (HS and LS), scratching levels did not differ between males (median rate per minute 0.213; interquartile range 0.10–0.30) and females (median rate per minute 0.151; interquartile range 0.10–0.23) (Mann–Whitney's test $U = 52.5$, $n_{\text{males}} = 9$, $n_{\text{females}} = 14$, $p = 0.508$) (prediction 2 not supported).

Aggression levels were higher in HS (median frequency per minute 0.140; interquartile range 0.07–0.14) than in LS (median frequency per minute 0.118; interquartile range 0.00–0.10) (Wilcoxon's test $T = 52.00$, ties $= 0$; $N = 23$ individuals, $p < 0.009$). Scratching levels were higher in HS (interquartile range 0.16–0.42) than in LS (interquartile range 0.07–0.16) (Wilcoxon's test $T = 13.00$, ties $= 0$; $N = 23$, $p < 0.001$) (medians reported in Fig. 2) (prediction 3a supported).

In both LS and HS, scratching was significantly lower after (interquartile range: LS 0.00–0.04; HS 0.00–0.07) than before/in absence of reciprocal grooming (interquartile range: LS 0.00–0.09; HS 0.09–0.13). The difference was significant for both LS (Wilcoxon's test $T = 125$, ties $= 7$; $N = 23$, $p = 0.003$) and HS (Wilcoxon's test $T = 246$, ties $= 1$; $N = 23$, $p = 0.000$) (medians reported in Fig. 3) (prediction 3b supported).

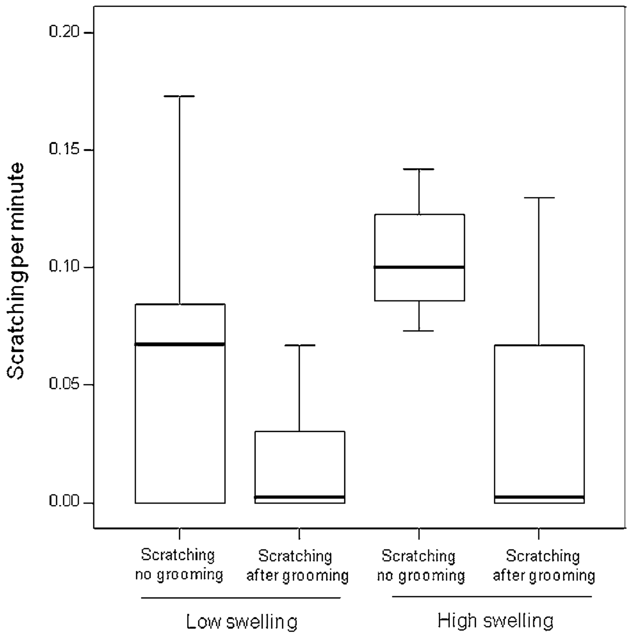

Fig. 3 Differences in the scratching (SCR bouts per minute) performed by animals following grooming and not following grooming, in both the low swelling (LS) and high swelling (HS) periods. Differences are significant ($p < 0.003$ for LS and $p < 0.001$ for HS). *Solid horizontal lines* medians, *box length* interquartile range, *thin horizontal lines* observed value range

Discussion

In our two *Lemur catta* groups, scratching levels (informing anxiety) did not correlate with ranking position (prediction 1 not supported). Estrus females perturb the relationship network and produce transient social instability, which we detected as changes in the hierarchical arrangement of both groups between the low swelling (LS) and the high swelling (HS) period (Table 1). Changes involved not only males, but also and most importantly females (in one group the alpha female lost the leadership in HS). Since social relationships in *Lemur catta* are built upon female hierarchy, the transient perturbation produced by estrus females can dramatically affect anxiety levels of all group members. Social unpredictability, here related to the mating period, is a nonselective, "blind" agent acting on the anxiety levels of both dominants, experiencing higher psychological stress because their ranking position is potentially at risk (Sapolsky 1983, 1992), and subordinates, especially due to the risk of renewed attacks (Aureli et al. 2002). As a result, the possible differences between subordinates and dominants with respect to anxiety levels are dampened or reduced, and cannot be detected.

Such social unpredictability could also account for the lack of sex difference in scratching levels, suggesting that males and females experience anxiety to a similar extent in the mating period (prediction 2 not supported). Certainly, anxiety in males is related to the aggression they receive from females, which reject copulation attempts, and by other males, which try to prevent competitors from accessing females (Jolly 1966; Sauther and Sussman 1993). Nevertheless, females experience high levels of anxiety as well. They engage in mate patrolling aggressions, either to repel particular males from attempting to copulate or to separate a male from another female during mounting (Sauther 1991; Sussman and Richard 1974). The high levels of both inter- and intrasexual competition, which add to social unpredictability, probably dampened sex differences (and consequently rank differences) in scratching levels during the mating period (Jolly 1966; Palagi et al. 2003, 2004b; Sauther 1991; Sussman and Richard 1974).

Social unpredictability, including competition within and between sexes, can also explain the increase of aggression in the high swelling (HS) period. The fact that aggression and scratching rates are maximal in HS suggests that anxiety increased along with mating-related conflicts (prediction 3a supported). In general, an increase of scratching levels can also derive from an increase in temperature and humidity (Ventura et al. 2005), but this was not the case in our study. In fact, temperature and humidity steadily decreased over the mating period (thus being lower in HS than in LS), and consequently, such environmental variables can be excluded as possible causes of the scratching increase observed in HS.

In mammals, anxiety can negatively affect reproductive behavior (Schumacher et al. 1989; Carter 1992; McCarthy et al. 1996; Douglas 2005; Veenema and Neumann 2008; Meyer-Lindenberg et al. 2011; Lovejoy and Barsyte 2011). In *L. catta*, the short estrus period coincides with the maximum levels of anxiety, which may limit reproductive potential. Hence, it is expected that lemurs adopt some mechanisms to cope with anxiety. One of the best mechanisms used by haplorhines to manage anxiety is social grooming (Schino et al. 1988). Here we show that, also in ring-tailed lemurs, scratching decreases after grooming in the mating period (prediction 3 supported; Fig. 3). Thus, our findings indicate that grooming could assist in reducing anxiety also in strepsirrhines.

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Verreaux's sifaka fur condition in the spiny forest of southern Androy

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Résumé

Les conditions du pelage des animaux peuvent représenter un moyen fiable et un indicateur non invasif pour comprendre l'état de santé d'une population et distinguer des segments différents de la même population. En 2008 nous avons effectué un recensement de sifaka (*Propithecus verreauxi*) dans les forêts riveraines de la réserve de Berenty (foret galerie et de transition de Malaza et forêt secondaire aux

espèces allochtones de Ankoba) et dans six portions de forêt épineuse qui sont inclues dans le domaine privé de Berenty. Nous avons relevé l'état du pelage des animaux selon trois conditions: fourrure intègre (niveau 1), fourrure faiblement endommagée (ponctuée par des petites zones, sans pelage, couvrant moins de 30 % du corps; niveau 2), fourrure fortement endommagée (pelage manquant sur une surface supérieure à 30 %; niveau 3). Nous avons aperçu seulement quatre sifaka au niveau de pelage 3 et, par conséquence, nous avons pu évaluer statistiquement les différences seulement entre les niveaux de pelage 1 et 2, en comparant forêt épineuse et les forêts riveraines, soi au niveau des groups d'animaux (n= 41) soi au niveau des zones recensée (n= 9). Même si avons détecté un nombre significativement plus haut de sifaka avec la fourrure faiblement endommagée dans le domaine épineuse, la nature et surtout l'entité du dommage indiquent que les conditions du pelage n'arrivent pas vraiment à différentier des segments distincts dans la population de sifaka de Berenty.

Introduction

An index of coat condition can be a non-invasive tool for tracking health and stress at the population level (Jolly, 2009a). In fact, pelage growth can be directly influenced by the proximate stimulus of light (acting through neuro-endocrine pathways), by the nutritional status, and indirectly by temperature and behavior (Ling, 1970). Two main functions of fur are a) insulation, which allows conservation of body heat, thus reducing energy expenditure and food requirements; and b) shielding, which protects day-active mammals from excessive heat load from solar radiation (Scott et *al.*, 2001; Kenagy and Pearson, 2000).

Here, we considered coat condition of *Propithecus verreauxi* (Verreaux's sifaka) as a possible indicator of the "health status" of animals in different habitats and investigated whether it could provide information on possible population stress in the poorly investigated spiny forest of south Madagascar.

The dry spiny forest of southern Madagascar is a thorny environment, both metaphorically and literally speaking. Listed as one of the 200 most important ecological regions in the world, it harbors the highest level of plant endemism in Madagascar (Elmqvist et *al.*, 2007). In spite of its importance, the spiny forest is underrepresented in terms of protection and conservation programs (Fenn, 2003; Ganzhorn et *al.*, 2003; Seddon et *al.*, 2000). To fill, at least in part, this gap, we investigated sifaka fur condition in different spiny forest parcels inside the Berenty Estate (Androy region, south Madagascar) and compared it with sifaka inhabiting the riverine forest areas inside the Berenty Reserve, a habitat much richer in staple food for lemurs.

Methods

Study site, survey technique, and fur condition evaluation

In March-April 2008, a comprehensive sifaka survey was conducted in the Berenty Estate, covering 134 ha of spiny forest and 60 ha of non-spiny forest areas. The Berenty Estate is located in the semi-arid Androy Region (rainfall averages less than 500 mm per year). The spiny forest is usually 3 to 6 m in height with dwarf and xerophyte plants, and emerging trees of the Family Didieraceae that may reach more than 10 m in height, such as the keystone species *Allouadia procera* (Elmqvist et *al.*, 2007). We performed the survey in all accessible spiny forest parcels (sacred areas, used as a cemetery, cannot be accessed by anyone except for local family clans) and in three riverine areas of the Berenty Reserve (on the Mandrare river), comprising a northern section (occupied by the 40 ha secondary forest of Ankoba dominated by the exotic legume species *Pithecellobium dulce*; S 24.99°; E 46.29°) and a southern section (Malaza: S 25.01°; E 46.31°) (Fig. 1 shows study locations). Inside Malaza we considered the 7 ha gallery forest (dominated by tamarinds; *Tamarindus indica*) and the front-transitional forest (13 ha) (Jolly et *al.*, 2006). In all the areas considered in this study, logging and hunting are prohibited, and the fossa (*Cryptoprocta ferox*) is absent.

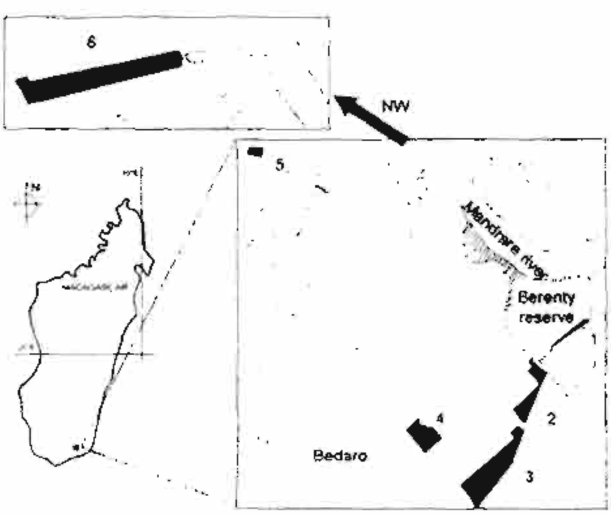

Fig. 1. Study site location: Berenty reserve (solid outline; white area: scrub; diagonal lines: Ankoba and Malaza riverine forests) and spiny forest fragments (black areas): 1 = Spiny Malaza; 2 = Spiny Reserve 1; 3 = Spiny Reserve 2; 4 = West Rapily; 5 = Fragment X; 6 = Anjapolo, about 13 km north-west of Berenty. Dashed outlines include degraded spiny and/or scrub areas. The rest of the territory (white) is covered by pasture and sisal fields. (Map based on Google Earth satellite view).

We performed the survey via walking, at a speed of about 1 km/h, along preexisting trails and through forest paths chosen *ad hoc* to have visibility of at least 50 m to the right and left (to avoid pseudoreplication we followed Norscia and Palagi, 2008).

During the census we evaluated the fur condition of each individual lemur. We scored coat condition on a 3-point scale: coat undamaged, with fur fully covering the body (level 1); ruffled coat, with fur punctuated by small areas of reduced/missing fur (on less than 30 % of the body, especially on elbows and/or knees; level 2); patchy coat, usually with black skin areas clearly visible due to reduced/missing fur (on more than 30 % of the body, especially on elbows/knees, external sides of forearms and thighs, fingers and toes; level 3) (Fig. 3).

Statistics

We performed the analyses at group or at forest site level. Owing to the small sample size ($n<10$ for forest sites) or deviation from normality (when n$10, in the analyses per group; Kolmogorov-Smirnov, $p<0.05$), we applied non-parametric tests (Siegel and Castellan, 1988) and considered exact p-values according to Mundry and Fischer (1998).

Results

In total we counted 183 sifaka adults and 25 infants (less than 1 y old, not included in the analyses). In the riverine forest areas we counted 81 adult males and 57 adult females whereas in the spiny forest we counted 45 individuals and were able to sex 21 adult males and 19 adult females. Overall, we observed level 3 fur condition only in four subjects (two in the spiny and two in the non-spiny forest areas). Thus, we considered only level 1 (undamaged coat) and 2 (moderately missing fur) for the analyses and found that the proportion of individuals with level-1 fur was significantly higher in the non-spiny than in the spiny areas both in the analysis per forest site (Exact Mann-Whitney U test, $n_{non-spiny}=3$, $n_{spiny}=6$, $Z=-2.35$, $p=0.024$) (Fig. 2) and in the analysis per group (Mann-Whitney U test, $n_{non-spiny}=31$, $n_{spiny}=11$, $Z=-3.26$, $p=0.001$).

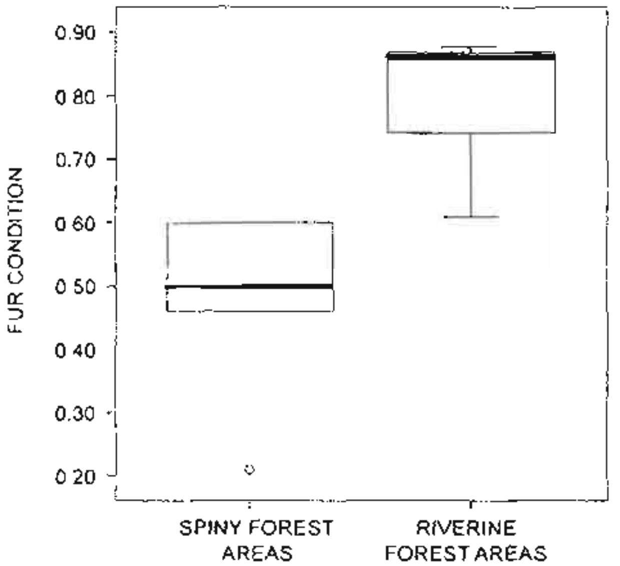

Fig. 2: Difference in fur condition (proportion of individuals showing level-1 fur) between the spiny and riverine forest areas. The difference is significant ($p<0.05$). Black lines: median; box: 25-75%; whiskers: non-outlier range.

Discussion

Monitoring coat condition in an apparently healthy population can yield a baseline of data for climate changes (being influenced by climatic factors such as temperature and amount of solar radiation) and eventual pathology, and reveal differences between population segments, and in forest fragments can track progressive degradation or improvement over time (Jolly, 2009a).

For the sifaka, we found that fur condition was better in the riverine forest areas than in the spiny forest domain (Fig. 2), reflecting the fact that riverine forest areas are richer than spiny forest areas in terms of staple food for lemurs. However, the comparison had to be restricted to individuals with full coat and moderately missing fur (level 1 and 2, respectively) because only four sifaka showed fur in truly bad condition (level 3; Fig. 3 shows the worst fur - and animal - condition observed in the forest).

In the *Lemur catta* of Berenty, serious fur loss was due to the alopecia syndrome, associated with the consumption of the toxic plant *Leucaena leucocephala* (Jolly, 2009b). Although consumed also by *P. verreauxi* in the past (Simmen *et al.*, 2003), *L. leucocephala* was not present in the spiny forest and removed from Berenty when this study was performed (H Rambeloarivony, pers. comm.).

Lemurs in the non-spiny forest domain are characterized by a good nutritional status (due to accessibility of protein-rich food) (Jolly *et al.*, 2006), which positively reflects on pelage. The sifaka of the spiny forest, which is characterized by an entirely open canopy, are exposed to high temperatures and need to save energy, and this situation positively influences pelage growth as well. Although different in nature, these two pressures (food availability and heavy exposure to light) both act positively on pelage, probably dampening the differences in fur conditions between spiny and non-spiny forest sifaka. Considering the type of coat "damage" in the spiny forest (ruffled fur and/or missing/reduced fur in small areas), the most likely and obvious correlate is the "unfriendly" vegetation, forming an open canopy that oblige the sifaka to travel through (and hide in) the thorny undergrowth.

Fig. 3: Sifaka male (probably old and/or sick) in the spiny forest showing fur in very bad condition (level 3). (Photo: Ivan Norscia)

Whilst sifaka density plummets when moving away from the riverine to the spiny forest areas, following the sharp gradient generated by water availability decrease (Norscia and Palagi, 2010), it seems that fur condition can only slightly differentiate sifaka segments of population inhabiting riverine forests and adjacent or close spiny forest areas.

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Rediscovery of Sibree's dwarf lemur in the fragmented forests of Tsinjoarivo, central-eastern Madagascar

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The recent genetic confirmation of a rare dwarf lemur species, *C. sibreei*, at Tsinjoarivo is bitter-sweet. The excitement of reporting the first known living population of this species is tainted by conservation concerns, as the forest fragment in which Sibree's dwarf lemurs were captured is highly disturbed and targeted for illicit logging. This species, like many others inhabiting rapidly degrading forests, faces the serious threat of extinction.

Taxonomic background of the genus, first field discovery, and subsequent recognition of *C. sibreei*

During the 19th century, the small nocturnal lemurs of Madagascar were clumped in a chaotic array of species and genera. For most of the 20th century, however, dwarf lemurs (*Cheirogaleus*) were classified in only two species: the eastern *C. major* and the western *C. medius* (Schwarz, 1931). Around the turn of the century, Groves (2000) conducted a taxonomic revision of the genus on the basis of morphological analysis of museum specimens and increased the species number to seven: *C. medius*, *C. adipicaudatus*, *C. major*, *C. ravus*, *C. crossleyi*, *C. minusculus* and *C. sibreei*. This last species, in fact, had been originally described by the Swiss naturalist Forsyth Major in 1896 during one of his expeditions to Madagascar (Forsyth Major, 1896). He had named it *Chirogale sibreei* in honor of fellow naturalist James Sibree, who had spent more than fifty years in Madagascar and had written extensively about its people, fauna, flora and geology. Forsyth Major published measurements of an individual "obtained from the neighbourhood of Ankeramadinika," a locality vaguely described by its discoverer as "one day's journey to the east of Antananarivo", but in fact a well-known village at the time, located in the central high plateau on the road that connected Antananarivo to Mahatsara on the east coast (Capitaine "X", 1901). In his taxonomic revision, Groves (2000) included as *Cheirogaleus sibreei* not only the holotype from Ankeramadinika (currently housed at the Natural History Museum in London) but also three additional specimens (3 skins and 1 skull), two of which came from Ampasindava, northwestern Madagascar, and one from an unclear provenance (Imerina, which refers to a region of the central highlands around Antananarivo).

The taxonomic shrinkage of *Cheirogaleus*

The increase in the number of species within the genus *Cheirogaleus* was not surprising because dwarf lemurs occupy a wide variety of habitats in Madagascar, and their close relatives, the mouse lemurs (*Microcebus*), had undergone a taxonomic explosion of their own with more than 10 species described during the past 15 years (Louis *et al.*, 2008; Olivieri *et al.*, 2007; Radespiel *et al.*, 2008). However, Groves' 2000 revision of dwarf lemur taxonomy did not escape criticism, not least of which had to do with the criteria that he used to define species, the lack of reliable locality information from museum specimens, and the absence of on-the-ground surveys to assess geographic boundaries and variation among species (Blanco *et al.*, 2009; Tattersall, 2007). A recent and more comprehensive revision of dwarf lemur taxonomy was carried out by Groeneveld and colleagues, who compiled genetic and morphometric data from field as well as museum specimens from a variety of localities across Madagascar, including some of the specimens studied by Groves (Groeneveld *et al.*, 2009; 2011). This research showed overall consistency between morphological and genetic data in recognizing only three *Cheirogaleus* species: *C. medius*, *C. major* and *C. crossleyi*. Individuals that previously had been assigned to *C. adipicaudatus* fell within the *C. medius* clade, and those named as *C. ravus* grouped with *C. major*. Results were inconclusive for *C. minusculus* and *C. sibreei* because holotype specimens were not available for sampling and their genetic affiliation could not be determined. Genetic data from one of the *C. sibreei* museum specimens from Ampasindava linked this specimen to *C. medius*. Nevertheless, the *C. sibreei* holotype from Ankeramadinika was larger and did not group morphologically with other *C. medius*. This suggested that the individuals from Ampasindava may have been misclassified by Groves as *C. sibreei* (Groeneveld *et al.*, 2010). The status of this species remained equivocal.

Second field discovery of *C. sibreei*, at last

The story of a dwarf lemur named "May" told by Mitchell Irwin (2002) turned out to be rather prophetic. Irwin's research team rescued this dwarf lemur badly burned in a

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La bouche ouverte, relaxée, est un signal ludique dans les makis à queue annelée en liberté

Introduction

Les signaux de jeu sont couramment utilisés par les animaux pour communiquer leur motivation ludique et pour limiter le risque que les actes ludiques les plus bruts soient mal interprétés par les compagnons de jeu. La bouche ouverte relaxée (*relaxed open mouth*) est l'expression de la face la plus fréquemment utilisée pendant le jeu par nombreux mammifères et représente la version ritualisée du mouvement qui anticipe une morsure de jeu. La nature de signalisation de cette expression a été prouvé dans de nombreuses espèces de singes mais jamais démontrée dans les prosimiens. Notre objectif a été d'évaluer si, aussi dans les prosimiens, la bouche ouverte relaxée possède une fonction de communication réelle. Nous avons étudié le *Lemur catta* en liberté, dans la forêt de Berenty (Sud Madagascar), une espèce qui est caractérisé par des habitudes très sociaux y compris des interactions ludiques intenses. Les lémuriens de cette espèce utilisent largement des signaux ludiques, principalement réalisées avec leur queue annelée. La fonction de signalisation de la queue (*tail play*) a été largement démontrée. Nous avons analysé à la fois les signaux de jeu avec la queue et la bouche ouverte au fin de vérifier comment leur distribution est affectée par différentes variables de jeu (par exemple la symétrie de la session ludiques, le nombre d’animaux qui jouent ou l'utilisation précédente du même pattern comportemental).

Résultats et Discussion

Le résultats montrent que les lémuriens utilisent la bouche ouverte relaxée comme un signal de communication pendant le jeu. La bouche ouverte était plus fréquente lors des interactions les plus débalancées caractérisées par une forte asymétrie dans les patterns effectués par les deux joueurs (offensive/neutre). Par rapport au jeu signalé à travers la queue, la bouche ouverte était plus fréquente pendant les interactions dyadique (deux joueurs) que pendant les interactions polyadiques (au moins trois joueurs) et, étant un signal très directionnel, il a été plus fréquemment reproduit par le compagnons de jeu. Par conséquent, la bouche ouverte doit être effectué pendant le jeu face-à-face de sorte que la détection du signal peut être optimisée. En conclusion, la bouche ouverte relaxée dans lémuriens, ainsi que dans des autres primates, semble être un signal rituel utilisé pour engager et, peut-être, soutenir et prolonger l'interaction ludique.

American Journal of Primatology

RESEARCH ARTICLE

Relaxed Open Mouth as a Playful Signal In Wild Ring-Tailed Lemurs

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Play signals are commonly used by animals to communicate their playful motivation and to limit the risk that rough acts are misunderstood by playmates. The relaxed open mouth is the most common facial expression performed during play in many mammals and represents the ritualized version of the movement anticipating a play bite. The signaling nature of this expression has been proven in many haplorrhine species but never demonstrated in strepsirrhines. Our purpose was assessing whether, also in strepsirrhines, the relaxed open mouth has an actual communicative function. We studied wild ring-tailed lemurs (*Lemur catta*), characterized by highly social habits including intense playful interactions. They largely use playful signals, mostly performed with the black and white tail. The signaling function of the tail (tail play) has been widely demonstrated. We analyzed both tail play and the relaxed open mouth to verify how their distribution is affected by different play variables (e.g., play session symmetry, number of play mates, previous use of the same pattern). Indeed, ring-tailed lemurs use the relaxed open mouth as a communicative signal during play. Relaxed open mouth was more frequent during unbalanced interactions showing the highest asymmetry in the patterns performed by the two players (offensive/neutral). Compared to tail play, relaxed open mouth was more frequent during dyadic than polyadic interactions and, as a highly directional signal, it was more frequently replicated by the play mate. Therefore, the relaxed open mouth needs to be performed face-to-face so that signal detection can be optimized. Similar to previous findings in monkeys and apes, the relaxed open mouth in lemurs seems to be a ritualized signal used to engage and, perhaps, sustain playful interaction. Am. J. Primatol.

Key words: play fighting; facial displays; playful signals; face-to-face interaction; *Lemur catta*

INTRODUCTION

Play is a social interaction featuring complex communicative signals [Fagen, 1981, 1993]. Social play, especially in its more vigorous form (e.g., play fighting), can imply a certain amount of risk for the players that have to attune their actions to avoid the degeneration of the interaction [Palagi, 2008, 2009; Pellis & Pellis, 1996, 1997]. Visual signals are generally used during ambiguous situations in which the playfulness of performers may be misunderstood by recipients: under such circumstances play signals are helpful in confirming that play is just play, thus avoiding the escalation into real aggression and prolonging the session [Bekoff, 2001; Mancini et al., 2013a; Palagi, 2008; Pellis & Pellis, 2009; Seyfarth & Cheney, 2000; Seyfarth et al., 2010].

Facial displays are a key for successfully managing playful bouts [Henry & Herrero, 1974; Mancini et al., 2013b; Waller & Dunbar, 2005]. Among facial signals, the relaxed open mouth (ROM) is certainly the most widespread across many mammalian taxa [Fox, 1970; Henry & Herrero, 1974; Jolly, 1966; Palagi, 2006; van Hooff & Preuscholft, 2003]. It has been hypothesized that the OM is the exaggerated and ritualized form of the movement that precedes the play bite, a very common behavioral pattern frequently used during play fighting [Andrew, 1963; Palagi, 2006; van Hooff & Preuscholft, 2003]. Through demonstrating the potential for aggression (in a caricatured overstated manner), the OM informs the recipient that the sender is actively avoiding aggressive behavior, confirming that the action is indeed only play. In carnivores, for example, playful

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In memory of Alison Jolly, the researcher who firstly understood the importance of studying lemurs to fully comprehend human evolution.

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facial signals seem to have an effective role in managing a playful interaction. Fox [1970] described the early development of play faces in gray, red, and arctic foxes as well as in coyotes and wolves, thus suggesting the importance of such signals in the ontogeny of play.

The OM is also present in almost all primate species, for this reason it is considered to be the most ancestral configuration of the playful facial displays in this taxon [van Hooff & Preuscholft, 2003]. The typical primate playful expression is the relaxed open mouth display (or play face) [van Hooff & Preuscholft, 2003]. In the play face, the mouth is opened in a relaxed mood with either the lower teeth or both the lower and the upper teeth exposed [Palagi, 2006, 2008]. The use of play face varies depending on the species, the context, the age/sex of the subjects, and other variables [Palagi, 2006, 2008; van Hooff & Preuscholft, 2003].

Most research on play signaling in primates has been devoted to monkeys and apes (haplorrhines). However, data from a wider array of primate taxa are needed for a more comprehensive understanding of the evolutionary roots of the behavior, its possible roles in play communication and the cognitive skills required for it [Armstrong, 1985]. Lemurs, which are relatively small brained, form an independent primate radiation and represent the most ancestral group-living primates [Tattersall, 1982]. Comparing lemurs to the better known haplorrhines is especially useful because these two distantly related primate groups share basic features of natural history. *Lemur catta* is a highly terrestrial and social species forming multimale/multifemale groups characterized by strong female dominance and male dispersal [Jolly, 1966].

Pellis & Pellis [1997] accurately described the dynamics of play fighting of a group of captive ring-tailed lemurs. They found that such dynamics strongly resembled those of real aggression, a result perfectly fitting with the competitive nature of the species. In a number of species, adult play fighting has been reported to be rougher and more likely to escalate into serious fighting than juvenile play fighting [Fagen, 1981; Pellis, 2002]. However, the low level of escalation (1.8%) found in adult lemurs, suggests that they are able to cope with possible ambiguous situations, with signals probably having a role in avoiding that playful interactions end up with serious fights [Pellis & Pellis, 1997]. Communication in *L. catta* mostly involves the use of the tail [Jolly, 1966]. However, despite the reduced mobility of face muscles [Fleagle, 1999; Jolly, 1966], ring-tailed lemurs can produce an open mouth display.

With its white and black rings, the tail of this lemur species has important roles in regulating many aspects of social life. The "stink fights" engaged by males during their agonistic interactions represent one of the most striking example of the importance of the tail in social communication [Jolly, 1966]. Adult males place their tails between the legs and upward in front of the torso, then anoint them with the secretions produced by specialized antebrachial glands. The "*anoint-tail*" action involves the performer rubbing the ventral surface of the wrist and forearm along his tail. Then, animals may repeatedly flick the tail downward over their head in order to spread the odor (*wave-tail*). During the agonistic wave-tail pattern, the male faces the opponent gazing at it, with its ears flattened against the top of the head. Mature males also anoint and wave their tails toward females as signals of appeasement or even submission during courtship [solicitation of copulation; Jolly, 1966; Pereira & Kappeler, 1997]. There is also a playful version of the communication pattern involving the use of the tail [Jolly, 1966]. Tail play (TP) has been widely described as a playful signal [Jolly, 1966; Palagi, 2009; Pellis & Pellis, 1997]. During play fighting, lemurs anoint their tails (TP) neither facing the playmate nor gazing at it. Infant lemurs begin to perform TP during the weaning period [at about 6 months of age; Palagi et al., 2002]. Tail use among playing ring-tailed infants and juveniles is often entirely anointing, rarely followed by waving.

Since in ring-tailed lemurs both the tail and the face seem to be involved in communicating the motivation to play [Palagi, 2009; Pellis & Pellis, 1997], *L. catta* is a good model species to understand if the open mouth is an actual play signal as tail play and how body and facial displays are used in order to regulate the social playful activity which, in this species, is characterized by a high level of competition. To explore the issue, we tested the following hypotheses.

The Ritualization Hypothesis for OM in *L. catta*

The process whereby expressive displays become ritualized and separated from their original function to serve a new function is defined as ritualization [Tinbergen, 1952]. Usually the relaxed open mouth (ROM) is considered a ritualized signal that simulates the intention of biting during a play session [Palagi, 2006; van Hooff & Preuscholft, 2003]. One of the first steps to evaluate if the OM is a communicative signal is demonstrating that its presence is not strictly linked to any specific behavioral pattern (in this case, the play bite). Therefore, if in ring-tailed lemurs OM has a communicative role, it should occur independently from the presence of a biting action (Prediction 1).

The Face-to-Face Hypothesis

It was demonstrated that TP is more frequent in polyadic than in dyadic playful interactions [Palagi, 2009]. This signal is highly visible at long distance and it is performed also without a direct face-to-face interaction between the players. In contrast, the OM efficacy in communicating the motivation to play strictly depends on face-to-face contact of the subjects [Mancini et al., 2013b]. In many primate species, the OM is considered a highly directional signal [Davila-Ross et al., 2008; Mancini et al., 2013b; Waller & Dunbar, 2005], thus we can hypothesize that in ring-tailed lemurs OM can be more detectable during those sessions characterized by a "face-to-face" interaction between the players and, therefore, more frequent during dyadic than polyadic sessions (Prediction 2a).

A facial display can tell a receiver something about the motivational state of the sender. Due to its interactive nature, a facial expression is only considered efficient when the receiver responds appropriately. For example, in humans (*Homo sapiens*) and in geladas (*Theropithecus gelada*) the probability that an infant will respond with a congruent facial expression to their mother, increases when she is attentive and smiling in humans [Jones et al., 1991], or producing a silent bared teeth expression in geladas [Mancini et al., 2013a].

Therefore, the correspondence between facial signals emitted and elicited could be a valuable criterion to evaluate the attentional state of playmates [Schmidt & Cohn, 2001]. If OM represents a more effective signal in dyadic sessions than TP, we expect that the former, more than the latter, can elicit a higher replication by the playmate (Prediction 2b).

The "Imbalance" Hypothesis

The estimate of balance/imbalance in a playful interaction is based on the directionality and amount of offensive or self-handicapping patterns during play. Play signals can avoid ambiguous situations from arising, by reducing the uncertainty of the contact and communicating the playfulness of a potentially dangerous act [Bekoff, 2001; Fagen, 1981; Loizos, 1967; Palagi, 2006]. If both TP and OM serve to fine tune play interactions, we would expect both to be sensitive to the degree of asymmetry within a dyad, the higher the degree: the higher the degree of asymmetry, the higher the frequency of TP and OM (Prediction 3).

The "Audience Effect" Hypothesis

Under some conditions, juvenile individuals should adjust the intensity of their play behavior or the frequency of their playful signals when particular adults are nearby [Flack et al., 2004]. For example, if a juvenile chimpanzee reduces the roughness of its play actions with a younger partner when the partner's mother approaches, he might be to avoid impending punishment by the mother of the play partner. Alternatively, an older juvenile might increase the frequency of play signals, such as play face, in order to communicate its own play motivation to the mother [Flack et al., 2004]. This fine-tuning in the behavioral activity according to the presence of particular bystanders is called "audience effect". If an audience effect is present, we would expect the presence of the mother of the youngest player to affect the frequency of both TP and ROM. Moreover, if TP is a signal that is particularly effective at longer distances, we would expect TP to be more sensitive to an audience effect than ROM signals (Prediction 4).

METHODS

Ethics Statement

This study was approved by University of Pisa (Animal Care and Use board). Since the study was purely observational the committee waived the need for a permit. The study was conducted with no manipulation of animals. The study was carried out in the private Reserve of Berenty (South Madagascar) and De Heaulme family (the owner) permitted us to observe animals. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates.

Study Location and Subjects

We conducted this study in the gallery forest of Berenty, a 200 ha reserve on the Mandrare River in southern Madagascar [for a complete description of the study site see Jolly et al., 2006]. In particular, this research was conducted in the northern part of the forest called Ankoba (24.99°S, 46.29°E), a 40 ha secondary forest 50–60 years old, with canopy at 10–15 m (except for a few emergent acacias to more than 20 m).

The study group of ring-tailed lemurs was composed of 16 habituated individuals, including 11 adults, two sub-adults, and three infants. Individual identification was based on sex, age, and on distinctive external features (size, missing fur patches, facial, and tail traits). To place each subject in the appropriate age class category we made use of body size, proximity of immature subjects to the mother, and sexual activity [Jolly, 1966]. We considered subjects as adults only if they were involved in sex-related behaviors.

Data Collection, Procedures, and Definitions

The daily observations, carried out by three observers (the last author and two field assistants), covered the period ranging from 6 am to 6 pm for 3 months, during the mating season (March–May 2011). Since each observer recorded the behavior of different sub-groups of animals, the data collected were independent. Data were tape-recorded and then entered into a computer database. We also video recorded (with the aid of a Panasonic Lumix fZ8) all play sessions during which animals were clearly visible and identifiable. Then, video-analysis was conducted with one-frame accuracy (one frame/ 10 msec) using Kinovea v. 0.7.10 software.

Data collection was preceded by a training period for the observers (the trainers were the first and the second author). During the training phase, the same focal animal was followed by the observers simultaneously, and the data were then compared. Training was considered complete when the Cohen's kappa (calculated for each of the behavioral patterns included in Table I) was higher than 0.70 [Kaufman & Rosenthal, 2009]. At weekly intervals, such procedure was replicated in order to control for the inter-observer reliability across the whole period of observation.

The presence of three observers concurrently working on the same group and the spatial cohesion of the animals allowed us to apply the all occurrences sampling method, otherwise impossible to be used in the wild. By all occurrences sampling [Altmann, 1974] we recorded every playful and aggressive behavioral interaction. For the playful events we collected the identity of both the initiator and receiver of the playful action, the number of the players involved, each playful pattern performed during play (see Table I for the definition of play patterns), the duration of the playful session and the identity of the animals in proximity, when present. For each aggressive encounter we recorded the identity of the aggressor and the victim, each behavioral pattern characterizing the aggressive event, and the outcome of the attack (decided, when it was possible to identify the loser and undecided, when it was not possible to distinguish the winner from the loser). By all occurrences sampling, we collected 208 play sessions (>10 sec), of which 149 sessions were video recorded.

A play session began when one partner invited another individual to play, or directed any playful pattern toward it (the playful patterns listed in Table I could be performed to invite to play). A session ended when playmates ceased their activities, one of them moved away, or when a third individual interfered, thus interrupting the interaction. If another play session began after a delay of 10 s, that session was counted as new. We collected the exact sequence of the behavioral patterns performed during each play session, starting from the play invitation of the initiator towards the playmate, until the end of the session.

In order to understand whether the relaxed open mouth (ROM) was a signal not necessarily linked to the pattern of playful bite, at the dyadic level we counted how many times an OM display was immediately followed by a bite and contrasted them with those in which OM display was not followed by a bite (Prediction 1).

To evaluate whether OM and TP distribution varied according to the number of players, we calculated the mean frequency of the signal (signal/ second) per each block of two (dyadic; A–B, B–C, A–C, etc.) or more players (polyadic; A–B–C, A–B–C–D, etc.) involved in the session (Prediction 2a). To estimate the probability of signal responsiveness we verified whether the receiver emitted a playful signal (ROM or TP) within a 5-sec time window after perceiving a previous stimulus (ROM or TP) emitted by the sender. We use a 5-sec criterion in order to be reasonably sure that the second signal was actually elicited by the previous one (Prediction 2b).

TABLE I. Behavioral Patterns Recorded During Play Fighting in the Study Group at Berenty (Madagascar)

Pattern	Physical contact (PC)–not physical contact (NPC)	Definition	Classification of the playful patterns
Acrobatic play (ACP)	NPC	One (solitary play) or more individuals (social play) climb, jump, and dangle from supports of the environment (i.e., branches)	Neutral
Grab gentle (GRG)	PC	An individual gently massages the playmate	Neutral
Play bite (PBIT)	PC	An individual bites a part of the playmate's body	Offensive
Play jump (PJ)	PC	An individual jumps with its four limbs in front of or on a playmate	Neutral
Play retrieve (PRE)	PC	An individual holds the playmate in order to prevent him from leaving the play session	Offensive
Play slap (PSL)	PC	An individual slaps any part of the fellow's body	Offensive
Play run (PRUN)	NPC	An animal rapidly following another	Offensive
Tail play (TP)	NPC	An animal rubbing the ventral surface of the wrist and forearm along the tail	Neutral
Relax open mouth (ROM)	NPC	An animal opening the mouth in a relaxed mood	Neutral
Supine position (SP)	NPC	An animal getting in supine position	Self-handicapping

To quantify the level of asymmetry of a session, we categorized the behavioral patterns including physical contact in offense and self-handicapping behaviors [Bauer & Smuts, 2007; Ward et al., 2008] (see Table I for the definition of play patterns). We calculated the asymmetry for each play session as follows [Ward et al., 2008 for domestic dogs]. The number of "wins" for individual A in a dyad equaled the number of offence behaviors by A directed at B plus the number of self-handicapping behaviors by B directed towards A. B's wins were calculated in the same way. Next, we calculated the proportion of wins for A as the number of wins for A divided by the number of wins for both A and B. We calculated the number of wins for B in the same way. We subtracted the smaller proportion of wins from the larger proportion to obtain a measure of the degree of asymmetry (Prediction 3).

Finally, in order to verify the existence of a modulation of signaling by the older playmate when the play bout occurred in proximity (within 5 m) of the younger partner's mother, we compared the frequency of each signal per dyad (signal/second) in the two following conditions: proximity (within 5 m) and not-proximity (more than 5 m) of the younger subject's mother (Prediction 4).

Statistical Analysis

The analyses involved individual lemurs, we employed nonparametric statistics [Siegel & Castellan, 1988]. Mann–Whitney *U*-test was applied to compare the overall frequency of ROM and TP between adult and immature subjects. The Wilcoxon's sign rank test was applied to compare the individual frequency of the two signals (ROM and TP). When the analysis involved dyads, we used randomization procedures to avoid pseudoreplication due to the non-independence of data (the same individual is included in more than one dyad, therefore dyads are not independent data-points). All the randomization tests were employed with a number of 10,000 permutations using the software Resampling Procedures 1.3 (David C. Howell, freeware). We applied the two-pair sample randomization test to compare the rates of ROM followed and not followed by a bite and to compare the rates of replication between ROM and TP. The two-pair sample randomization test was also applied to compare the frequency of ROM and TP in the two conditions: proximity and non-proximity of the younger player's mother. We applied the two-independent randomization test to compare the overall frequency of ROM and TP between dyadic and polyadic sessions.

Due to variable deviation from normal distribution, we ran two sets of Generalized Linear Mixed Models (GLMM, via SPSS 20.0) in order to examine the effect of different factors on either ROM frequency or TP frequency (dependent variables). The duration of the play session (sec) was introduced as a weighted variable. Play sessions with at least one event of OM or TP were entered in the test. Both dependent variables were distributed according to an inverse Gaussian function (Anderson–Darling, ns; EasyFit 5.5 Professional). Therefore, we ran the GLMM by selecting the inverse Gaussian distribution function with Power (−2.0) link function. We used robust estimation to handle violations of model assumptions and Satterthwaite approximation due to the less structured forms of covariance. Since age, sex, and rank variables correlated (correlation test via Resampling Procedures, $0.172 < r < 0.798$; $0.001 < P < 0.05$), these were combined into a single interaction variable (sex × rank × age). In the two tests, the identity of playmates (a personal code was assigned to each subject) was entered as random factor (nominal variable). We tested models for each combination involving the variables of interest (Table II), spanning from a single-variable model to a model including all the fixed factors (full model). To select the best model, we used the Akaike's Corrected Information Criterion (AIC_c), which corrects the Akaike's Information Criterion (AIC) for small sample sizes. As the sample size increases, the AIC_c converges to AIC. The model with the lowest value of AIC was considered to be the best model.

We carried out hierarchical rank order analysis with the aid of MatMan 1.0 Software by Noldus® [De Vries, 1993] using all dyadic decided agonistic interactions recorded during the observation. We

TABLE II. Description of the Variables Used in GLMM Analyses

Name	Type
Dependent variables	
Frequency of open-mouth (OM)	Continuous
Frequency of tail-play (TP)	Continuous
Fixed explanatory variables	
Individual characteristics	
Sex combination[a]	Ordinal (0 = male–male; 1 = male–female)
Age combination	Ordinal (0 = immature–immature; 1 = immature–adult; 2 = adult–adult)
Rank distance	Categorical
Play session characteristics	
Asymmetry degree	Categorical
Random variables	
Player 1	Nominal
Player 2	Nominal

[a] We did not record any female–female session.

calculated the improved index of linearity (h') and tested it by means of a randomization test with the aid of MatMan [Appleby, 1983; De Vries, 1995]. In order to get the rank order consistently with the linear hierarchy of the individuals, we used the "Reorder Matrix to Fit Linear Hierarchy" [option from the MatMan software—see De Vries, 1998] that revealed the rank order position for each individual. Using the dominance linear order we could calculate the rank distance between subjects.

RESULTS

Compared to tail play (TP), the relaxed open mouth (ROM) was more frequently performed (exact Wilcoxon's matched-pairs $T = 0$, ties $= 1$; $N = 11$; $P = 0.002$) by the 11 subjects which engaged in playful sessions (Fig. 1). Neither TP (exact Mann–Whitney $U = 9$, $N_{\text{infants}} = 5$; $N_{\text{adults}} = 6$; $P = 0.327$) nor ROM (exact Mann–Whitney $U = 12$, $N_{\text{infants}} = 5$; $N_{\text{adults}} = 6$; $P = 0.662$) differed as a function of the age class of subjects (Fig. 1).

The Ritualization Hypothesis for ROM in *L. catta* (Prediction 1)

The percentage of ROM not followed by a bite was 75.00% ±27.49 SE. The rates of ROMs (ROM/sec) not followed by bites were more frequent than those followed by bites (randomization two paired samples: $t = 3.025$; $N = 27$ dyadic sessions; $P = 0.006$; Fig. 2) (Prediction 1 supported). Only those dyadic sessions characterized by the presence of ROM were considered for this analysis.

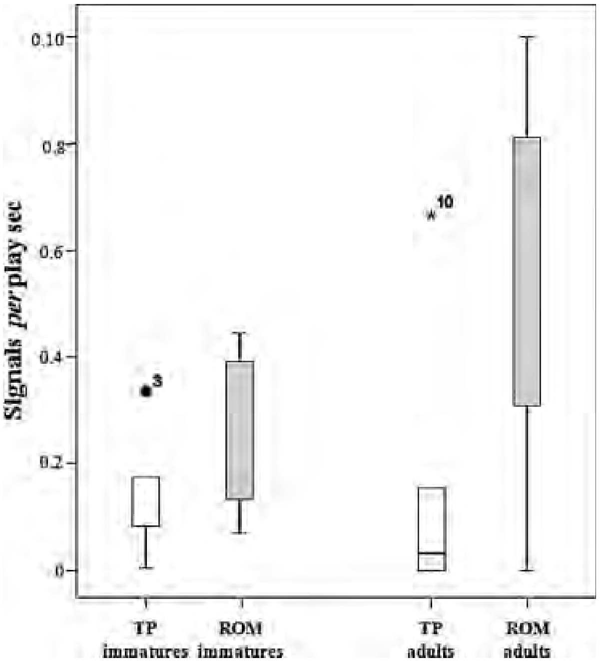

Fig. 1. Frequency of tail play (TP) and relaxed open mouth display (ROM) performed per play second by immature an adult subjects of the study group. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. Open dots indicate outliers more than 1.5 × IQR from the rest of the scores. Asterisks indicate outliers more than 3.0 × IQR from the rest of the scores.

The Face-to-Face Hypothesis (Prediction 2)

Dyadic and polyadic sessions were characterized by similar frequencies of TP (randomization two-independent samples: $t = -0.741$, $N_{\text{dyadic}} = 39$ sessions; $N_{\text{polyadic}} = 35$ sessions; $P = 0.479$); on the other hand, ROM occurred more frequently during dyadic than polyadic sessions (randomization two-independent samples: $t = -2.197$, $N_{\text{dyadic}} = 39$ sessions; $N_{\text{polyadic}} = 35$ sessions; $P = 0.022$; Fig. 3) (Prediction 2a supported).

Compared to TP, ROM was more frequently replicated (<5 sec) by the playmate (randomization two paired samples: $t = 3.441$; $N = 11$; $P = 0.009$; Fig. 4) (Prediction 2b supported). For this analysis, only those dyads ($n = 11$) showing both kinds of playful signals were included.

The "Imbalance" Hypothesis (Prediction 3)

Via General Linear Mixed Model (LMM) we verified which variables could explain the frequency of ROM and TP during dyadic sessions. Sex, age, and rank combination, and the degree of asymmetry were entered as fixed factors and the identity of the players were entered as random factors (Table II). This analysis involved only those play bouts which lasted more than 10 sec. As for the ROM frequency, only the asymmetry degree remained in the best model (best

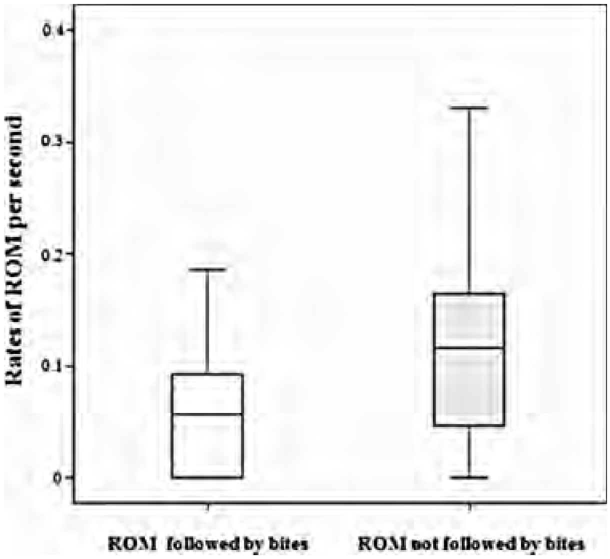

Fig. 2. Frequency of ROM per play second followed by a bite compared to ROM not followed by a bite. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR.

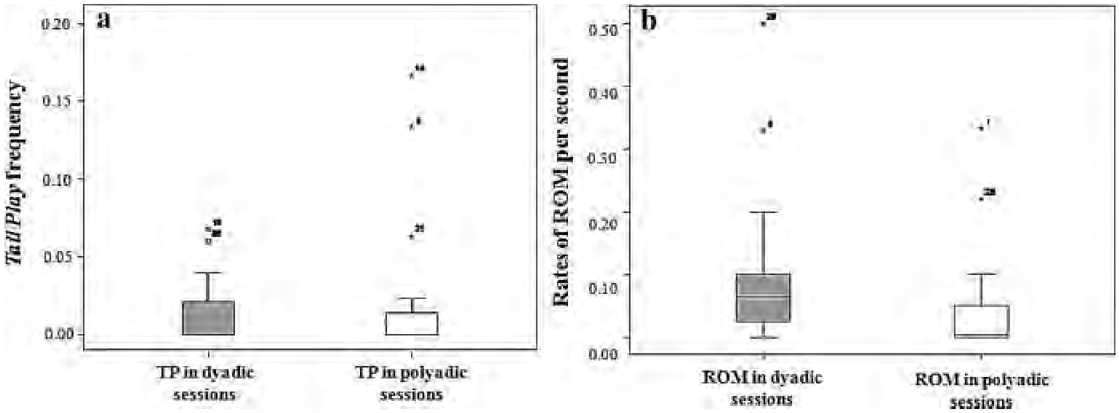

Fig. 3. Median frequency of TP (**a**) and ROM (**b**) per play second performed in dyadic ($N = 39$) and polyadic ($N = 35$) interactions. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. Open dots indicate outliers more than 1.5 × IQR from the rest of the scores. Asterisks indicate outliers more than 3.0 × IQR from the rest of the scores.

model $AIC_c = -272.690$; worst model $AIC_c = -207.593$). The frequency of ROM increased alongside the asymmetry degree of the playful sessions (Table III; Fig. 5). As for the TP frequency, the best model (best model $AIC_c = -74.971$; worst model $AIC_c = -68.709$) included the asymmetry degree, even though this factor did not significantly affect the frequency of the dependent variable (TP, Table III) (Prediction 3 partially supported).

The "Audience Effect" Hypothesis (Prediction 4)

To verify whether an audience effect (the presence of the infant's mother) had a larger impact on TP than ROM, we selected only those dyads involving infants playing with juveniles or adults (size mismatched pairs). The presence of the mother did not increase the frequency either of TP (randomization two paired samples: $t = 1.772$, $N = 8$, $P = 0.249$) or ROM (randomization two paired samples: $t = -0.094$, $N = 8$, $P = 0.938$). Surprisingly, TP was not more frequent than ROM either in the presence (randomization two paired samples: $t = -1.933$, $N = 8$, $P = 0.124$) or in absence of the mother (randomization two paired samples: $t = -2.039$, $N = 8$, $P = 0.059$) (Prediction 4 not supported).

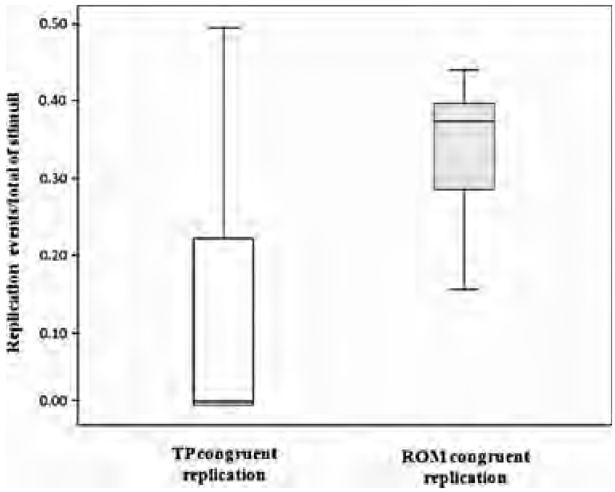

Fig. 4. Frequency of congruent replication per each triggering event perceived (TP → TP; ROM → ROM). Only congruent responses occurred within a 5-sec time-window after the perception of the triggering stimulus were considered.

DISCUSSION

Here, we provided quantitative data showing that lemurs signal their playful motivation not only by manipulating their black and white tail [tail play, TP—Jolly, 1966; Palagi, 2009] but also performing the relaxed open mouth display (ROM), which leads us to conclude that the ROM is an actual play signal.

In wild ring-tailed lemurs, ROM was mainly performed in the absence of subsequent biting, thus suggesting that the facial display represents a ritualized behavior (Prediction 1 supported). Pellis & Pellis [1997] studying the play fighting dynamics of a group of captive ring-tailed lemurs, found that ROM was used as a communication signal only in the 20–25% of cases during the pre-reproductive period [September–October in the northern hemisphere; Jolly, 1966]. Our data show that wild lemurs in Berenty are much more reliant on this communicative pattern during playful interactions (about 75% of cases), especially in the mating season [April–May in the Southern hemisphere; Jolly, 1966] a period characterized by a high level of competition and aggressiveness [Palagi et al., 2003, 2004]. Ring-tailed lemurs are a highly seasonal species, whose social behavior changes according to the photoperiodic and temperature variations [Vick & Pereira, 1989].

TABLE III. Best GLMM Explaining the Frequency of ROM ($AIC_c = -272.690$) and TP ($AIC_c = -74.971$)

	Df1	Df2	F	Significance level
Relaxed open mouth (ROM)				
Fixed factors				
Asymmetry degree	2	121	12.507	<0.001
Random factors	Variance	SE		
Player 1 identity	0.000	0.000		
Player 2 identity	0.000	0.000		
Tail play (TP)				
Fixed factors				
Asymmetry degree	2	62	0.411	0.665
Random factors	Variance	SE		
Player 1 identity	0.000	0.000		
Player 2 identity	0.001	0.001		

Note: df, degrees of freedom; SE, standard error.

During the mating season both males and females increase their levels of aggression and competition thus showing extremely low levels of tolerance towards group members [Jolly, 1966; Palagi et al., 2003, 2005; Sclafani et al., 2012]. Probably, compared to other phases of the year, playing during the mating season is more risky and a redundancy of clear playful signals is required in order to limit the possibility of escalation. Redundancy in play signals, both in terms of amplification and frequency, has been described in many mammalian species which show high levels of aggressiveness in their social relationships [coyotes, Bekoff, 1974; hyenas, Drea et al., 1996; bears, Henry & Herrero, 1974]. Moreover, in contrast with Pellis and Pellis, our sample included infants, juveniles, and adult subjects that frequently played together in mixed-age combinations. The differences in the animal sample, in timing of observations and environmental conditions (wild vs. captivity) could explain the different percentages of ROM revealed by the two studies.

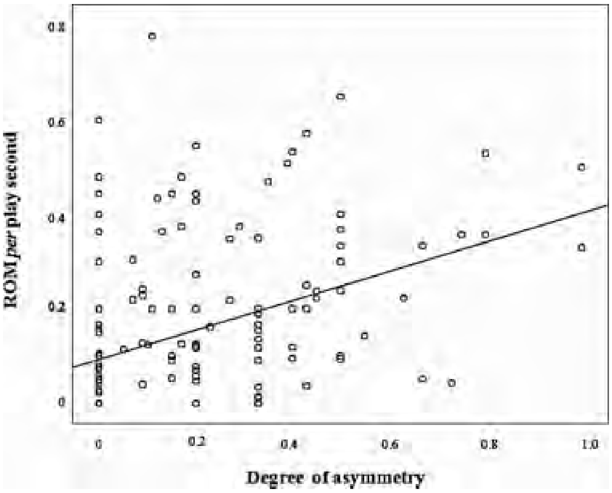

Fig. 5. Scatterplot showing the variation of ROM frequency per play session according to the asymmetry degree. Asymmetry degree of the play sessions varies from 0 (complete symmetry) to 1 (complete asymmetry).

The comparison of the frequency of the two signals (relaxed open mouth, ROM and tail play, TP) according to the number of players revealed an interesting result. While TP did not show any difference in dyadic and polyadic sessions, ROM was more frequent in dyadic than in polyadic interactions (Prediction 2a supported). When a playful facial signal is combined with facial expressions that are not play-specific, such as the relaxed open mouth combined with teeth-baring during play fighting in *Macaca tonkeana*, an animal may continue displaying this signal once the playmate has withdrawn from the playful contact [Pellis et al., 2011]. However, as a play-specific facial expression, ROM should achieve its best potential when performed during face-to-face interactions, as it frequently occurs in dyadic play fighting. There is evidence that different animals, other than great apes and humans, can choose appropriate forms of communication depending on another animal's attentional state [Horowitz, 2009]. For example, a typical canine play signal (e.g., play bow) may go unobserved if the other animal is not oriented toward the signaler. Only when a dog has gotten the attention of another dog, she/he directs visual play signals toward it. In this view, the association between play bow and attention-getting behaviors strongly indicates the cognitive foundations of signal production. Further support for the "face-to-face interaction" hypothesis comes from the response of receivers. In our study, the analysis on signal responsiveness showed that ROM was more frequently replicated by the playmate than TP (Prediction 2b confirmed). Facial responsiveness (or facial replication) allows animals to detect contingencies in their social world, to synchronize their activity, and to learn the context in which an action can be performed [Palagi & Mancini, 2011; Provine, 1996, 2004]. Therefore, a ROM display in lemurs has to be performed facing the playmate so that the signal can reach its maximum efficacy. It does not seem that lemurs make use of specific attention-getting behaviors while playing, even though it seems that they are able to optimize the use of facial displays. On the contrary, TP can be performed even without a face-to-face contact between the playmates and also when they are separated by several meters. Therefore, TP seems to be characterized by less directionality than ROM and, consequently, also by a lower level of replication by the receiver.

The degree of asymmetry of the session significantly affected the distribution of ROM, but it did not have a significant effect on the distribution of TP (Prediction 3 partially supported). ROM was more frequent when the players engaged in more unbalanced interactions, thus suggesting that signaling the benign intent is particularly useful when the session implies a greater amount of risk [Bekoff, 2001; Silk, 1997]. We suppose that the number of signals performed in a play session is directly dependent on the way in which animals play, and particularly on the number of offensive behaviors performed during play. Contrary to the less directional TP, ROM could be more efficient in decreasing the probability of an escalation of the playful interaction [Palagi, 2008, 2009; Pellis & Pellis, 1996, 1997]. Indeed, the higher level of replication characterizing ROM underlines the importance of the interactive nature of the signal which can favor the behavioral synchronization between players. ROM can be viewed as a buffering display which, through a retroactive mechanism [Pellis & Pellis, 1996], can restore some degree of balance to a play bout.

Finally, we did not find support for the "audience effect" hypothesis (Prediction 4 not supported). Both TP and ROM were not affected by the presence of the mother of the youngest playmate. More specifically, we expected that TP, being a highly visible signal, was more useful than ROM in manipulating the social context in which the play session occurred. However, we did not find any evidence that TP of the older playmate was affected by the presence of the younger partner's mother more than ROM.

In contrast to chimpanzees, a relatively large brained species, who use their playful signals in a cognitive and manipulative way [Flack et al., 2004], lemurs seem not to be able to face such kinds of cognitive challenges.

In conclusion, our work has added a new piece to the puzzle of play communication in *L. catta*. It seems that not only are lemurs able to communicate the motivation to play through signals, including ROM, but also that they can select the proper signal (a facial expression, ROM or a body display, TP) as a function of the type of play they engage in. The next step will be to understand if ROM and TP have not only different, but also complementary roles in managing playful interactions. To accomplish this issue an accurate sequential analysis on the response of the receiver immediately after perceiving the signal is required.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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La saison de la paix: la réconciliation dans une espèce despotique (*Lemur catta*)

Introduction

Cependant despotique un groupe social peut être, la gestion des conflits d'intérêt est crucial pour préserver les avantages de la vie de groupe, principalement basées sur la coopération. Dans les groupes despotiques, la gestion post-conflictuel peut être effectuée à travers la réconciliation (définie comme la première réunion, avec un contact affiliatif, entre les adversaires après d'une agression), même si les taux de conciliation peuvent varier considérablement. *Lemur catta* est défini comme une espèce despotique parce que les groupes sont caractérisés par une hiérarchie stricte et linéaire maintenue par les femelles adultes (le sexe dominant), principalement à travers de agressions. La présence de réconciliation a été détectée seulement dans un des quatre groupes en captivité de *L. catta* étudiés dans le passé. Nous considérons ici les variables qui influent sur l'apparition de la réconciliation dans huit groupes despotiques de *L. catta* (cinq dans la forêt de Berenty, Madagascar; trois hébergés au zoo de Pistoia, Italie)

Résultats et Discussion

Nous avons analysé 2339 paires de PC-MC (*Post-Conflit et Matched Control*). Puisque *L. catta* est caractérisée par une dominance féminine très forte, mais il y a aussi la formation de coalitions entre femelles, nous nous attendions à détecter la présence de la réconciliation dans les espèces étudiées. Constamment, nous avons trouvé la réconciliation dans un groupe en captivité et deux groupes en liberté, offrant ainsi la première preuve de la présence de réconciliation dans l'espèce d'étude à l'état sauvage.

En outre, comme cette espèce est caractérisée par une reproduction saisonnière (l'accouplement se produise une fois par an), nous nous attendions à des fluctuations saisonnières des niveaux de réconciliation. À travers d'une analyse GLMM en utilisant les données de tous les groupes de Berenty et d'un groupe en captivité suivi pendant plus d'une année, nous avons constaté que la saison (mais pas le rang, l'identité de groupe, ou l'identité, le sexe et l'âge des individus) affectées de manière significative les taux de réconciliation individuelle, et ces taux étaient les plus bas au cours de la période d'accouplement.

Ainsi, la réconciliation peut être présent dans des groupes dans lesquels les individus dominants limitent fortement les relations sociales, sauf quand les avantages de la coopération intra-groupe sont surmontés par la compétition, comme cela qui se produit dans des reproducteurs saisonniers pendant la période des accouplements. Nous concluons que dans les groupes sociaux despotiques dans lequel des coalitions sont observés, la bonne question n'est pas *si*, mais *quand* la réconciliation peut être présent.

RESEARCH ARTICLE

The Season for Peace: Reconciliation in a Despotic Species (*Lemur catta*)

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Abstract

However despotic a social group may be, managing conflicts of interest is crucial to preserve group living benefits, mainly based on cooperation. In despotic groups, post-conflict management via reconciliation (the first post-conflict reunion between former opponents) can occur, even if conciliatory rates are considerably different. *Lemur catta* is defined as a despotic species because groups are characterized by a strict linear hierarchy maintained by the adult females (the dominant sex) mainly via aggression. Reconciliation was reported in one out of four captive groups of *L*. *catta*. Here we investigate which variables influence the occurrence of reconciliation in these despotic groups. We analyzed 2339 Post Conflict (PC)-Matched Control (MC) observation pairs, collected on eight groups (five in the Berenty forest, Madagascar; three hosted at the Pistoia Zoo, Italy). Since *L*. *catta* is characterized by steep female dominance but shows female-female coalitionary support, we expected to confirm the presence of reconciliation in the study species. Consistently, we found reconciliation in one captive group and two wild groups, thus providing the first evidence of the presence of this phenomenon in wild *L*. *catta*. Moreover, because this species is a seasonal breeder (with mating occurring once a year), we expected seasonal fluctuations in reconciliation levels. Via a GLMM analysis using data from all wild groups and on a captive group followed for more than one year, we found that season (but not rank; individuals' identity, sex, and age; or group identity) significantly affected individual reconciliation rates, and such rates were lowest during the mating period. Thus, reconciliation can be present in groups in which dominants strongly influence and limit social relationships (steep dominance hierarchy) except when the advantages of intra-group cooperation are overcome by competition, as occurs in seasonal breeders when reproduction is at stake. We conclude that in despotic social groups in which coalitions are observed, the right question is not *if* but *when* reconciliation can be present.

Introduction

The management of conflicts of interest is crucial to preserve group living benefits, even in despotic societies. In these kinds of societies, to preserve social integrity, violence is minimized via the strict control exerted by dominants over other individuals ("negative peace", *sensu* Galtung [1]). Yet, in humans and other social mammals, dominant individuals or subgroups may need the support of others to obtain resources and maintain the *status quo* [2–7]. Consequently, strategies of mutual help other than competition for dominance and resources must be enabled, such as cooperative breeding, hunting, and coalitionary support during between-group conflicts [8–11].

Reconciliation or peace-making, defined as the first affinitive contact between former opponents occurring within few minutes after the conflict, is one of the main mechanisms to manage conflicts [12]. The phenomenon is present in social animals, including a bird species (e.g. ravens, *Corvus corax* [13]), various non primate mammals (e.g. domestic goats, *Capra hircus* [14]; dolphins, *Tursiups troncatus* [15]; domestic dogs, *Canis lupus familiaris* [16]; horses, *Equus caballus* [17]; red-necked wallabies, *Macropus rufogriseus* [18]), and human and non human primates (*Homo sapiens* [19]; chimpanzees, *Pan troglodytes* [20], [21]; bonobos, *Pan paniscus* [22]; *Gorilla beringei* and *Gorilla gorilla* [23–25]; wild macaques, *Macaca* spp. [26, 27]; captive guereza, *Colobus guereza* [28]; captive patas monkeys, *Erythrocebus patas* [29]; captive squirrel monkeys, *Saimiri sciureus* [30]; captive white-faced capuchins, *Cebus capucinus* [31]).

By restoring the relationship between former opponents [32–39], reducing the probability of further fights [23], [33], [34], [40–45] and/or reducing anxiety in the victim [21], [46–50], reconciliation is crucial to preserving social unity from the disruption caused by uncontrolled conflict spreading in the group. Therefore, reconciliation is expected to be present any time that it is valuable for the group members (including dominants) to preserve the alliances that facilitate group survival, thus preserving the benefits of group living [51].

Consistently, reconciliation has been found also in species with a despotic dominance style [5; 52–55]. According to the definition of Flack and de Waal [52], in despotic groups dominance dyadic asymmetries remains quite stable over time because they reinforced through severe aggression. Instead, in tolerant groups dyadic asymmetries can exist but many relationships are unresolved. Examples of animals living in despotic groups and that are able to reconcile include spotted hyenas (*Crocuta crocuta* [53]), wolves (*Canis lupus lupus* [5]), Japanese macaques (*Macaca fuscata* [54]), and wild chacma baboons (*Papio ursinus*: Cheney, Seyfarth & Silk [55]). Similar to these species, *Lemur catta* can be defined as despotic because groups are characterised by a linear and steep hierarchy with clear-cut dominance relationships [56]. Females are dominant and their dominance is maintained also through severe aggression by dominants over subordinates [56–63]. In this species, the presence of reconciliation was found in one out of four captive troops in which post-conflict management was studied [64],[65].

The linkage between reconciliation and the level of authoritativeness (or despotism) has been qualitatively examined in humans, with friendly peacemaking being favored by minimal authority (power exercised over others; [66]). The linkage between reconciliation and dominance style has been also quantitatively assessed in tolerant to despotic macaque species ([52], [67]), with tolerant species (e.g. Tonkean macaques, *Macaca tonkeana* [37],[68], [69–71]) showing higher reconciliation levels than despotic species (Japanese macaques, *Macaca fuscata* [54]). The same linkage has been hypothesized in strepsirrhine primates [64], which can also show more or less mild and flexible dominance hierarchies [56]. In this primate taxon, reconciliation was indeed found in species with more relaxed (i.e. less steep or transitive) dominance relationships (captive *Eulemur* wild *Eulemur rufusxcollaris* [45]) *rufus* [64], [72]; wild *Propithecus verreauxi* [73]) but not in captive *Eulemur macaco* showing strong female dominance [72].

In the present study, we investigate the factors that can explain the occurrence of reconciliation (or lack thereof) in different captive and wild groups of *L. catta* and make inferences about the conditions that favor the presence of reconciliation in despotic groups. As a primate species belonging to the group (strepsirrhines) that diverged from the common ancestor some 60 million years ago [74], *L. catta* also offers the possibility to make inferences about the biological roots of peace-making dynamics found in humans and all other primates. For this investigation, we analyzed the data collected on the focal species both in the wild and in captivity across more than a decade to verify the following predictions:

Prediction 1

Similar to wolves and hyenas [5], [75], [76] *L. catta* is characterized by rigid hierarchy and high competition levels [57–63], [77–79]. Analogous to ring-tailed lemur troops, packs (in the case of wolves; [80]) and clans (in the case of hyenas [81], [82]) strictly defend their territories by directing severe aggression towards potential immigrants. Finally, although in a more limited form compared to canids and hyenids, *L. catta* females (the dominant sex in this species) are able to form coalitions, especially against other females, to preserve their dominance status or to gain the possibility to use a territory [10]. These traits led us to predict that, as in other despotic but cooperative species [58], reconciliation may be present in *L. catta* not only in captivity but also in the wild.

Prediction 2

In the animals breeding once or twice in the year, seasonality strongly affects social behaviour and competition levels [83]. Majolo & Koyama [84] found that in the population of despotic *Macaca fuscata* from Yakushima Island reconciliation levels changed seasonally. As most lemur species, *L. catta* lives and has evolved in a highly seasonal environment [61], [85,86] and is a seasonal breeder [58]. In fact, females are receptive once a year [87–89] and the mating period (from three weeks to two months depending on the site and the definition; see also: [57], [58], [90], [91]) is characterised by high competition and low affiliation levels. During the mating period, competition within and between sexes is extremely high and affiliation levels are low [58], [77], [92], [91]. Therefore, we expected that in *L. catta* seasonality would particularly affect reconciliation levels.

Methods

Ethics statement

Since the study was purely observational the Animal Care and Use board (University of Pisa) waived the need for a permit. The study was conducted with no manipulation of animals. The study was carried out in the private Reserve of Berenty (South Madagascar) and at the Pistoia Zoo (Pistoia, Italy). De Heaulme and family, owners of Berenty and Mr Cavicchio, owner and director of the Pistoia Zoo, permitted us to observe animals.

Study species

Lemur catta (ring-tailed lemur) is a cathemeral species characterized by seasonal fluctuations in olfactory behavior, group dispersal, tolerance level, and reproduction [58], [78], [79], [93–97]. *Lemur catta* has a steep, consistent, highly transitive and cohesive hierarchy (*sensu* Norscia and Palagi [56]), with females dominant over males [58], [59], [78], [98–99]). Male hierarchy is unstable, and at times, non-transitive, and both female-female and male-male dominance hierarchies are fluid and can change over time [100–102].

The mating season overlaps among the different groups of a population and can last from three weeks to two months (depending on the site, the year, the definition of mating period; [57], [58], [90], [91], [103]. However, the onset of the mating period varies between groups, and the whole mating season for the lemur population spans up to four months [57],[103]. Females experience an annual estrus of a few hours to days, and receptivity lasts 10–24 h after which the estrus period ends [58], [87]-[89], [59]. A second or third belated estrus is possible [58], [78], [79]). *Lemur catta* females have a visible estrus, which may be asynchronous with other females in their group [104]. The mating period starts about one month before copulations, when female perineal area starts to enlargen and the center of the genitalia becomes larger and pinker: this period of swelling anticipates estrus [58], [87]. Generally, receptivity coincides with the last day of maximal pink coloration of vaginal labia ([87], [103].

Study location and subjects

Berenty (Madagascar). We conducted this research on wild lemurs in the gallery forest of Berenty, a reserve on the Mandrare River in Southern Madagascar (for an extensive description of the forest, see [105]). Data collection was conducted in the northern part of the forest called Ankoba (S 24.998; E 46.298), a 40-ha secondary forest 50- to 60-years-old, with canopy at 10–15 m (except for few emergent acacias to more than 20 m) and high lemur density [105]. Observations were carried out in the periods November 2006-February 2007, April-July 2008, and March-April 2011 on five troops of *L. catta*. Details on group composition and observation periods are reported in Table 1. Kin relationships among group members were unknown but groups at Berenty (and other sites) are largely female matrilines (including sibling and offspring of the alpha female [10],[59], [106], [107]. The individuals were well habituated to the presence of humans. As in previous studies, individual identification was based on sex and distinctive external features [56–58].

Pistoia Zoo (Italy). We studied three captive troops (here named A, B, and C) at the Pistoia Zoo (Italy) in the periods February-May 1999, November 2003-February 2005. Details on group composition and observation periods are reported in Table 1. The captive groups were largely composed by the alpha female and kin (siblings and offspring of the alpha female). The lemurs were housed in an outside grassy enclosure (98 m^2). In 1999, groups A and B were kept in two separated indoor halls on the coldest days of the year (A: 10 m^2 indoor facility; B: 20 m^2 indoor facility). Large glass windows in the two indoor facilities allowed the lemurs to follow the natural day-light 24-h cycle. Each group utilized the outside enclosure for 4–6 h per day, separately. In 2003–2005, another group (C_c) was hosted at the zoo and could use the indoor facility previously used by the other groups (not present anymore). The observations took place outdoors and lasted from the end of October 2003 to February 2015. As in the wild and in previous studies at Pistoia Zoo, individual identification in captivity was based on sex and distinctive external features [57] [65], [73], [74].

Table 1. Composition of wild and captive groups, observation n periods and study sites.

Group	Observation months	Period	$Males_{adult}$	$Females_{adult}$	$Males_{juvenile}$	$Females_{juvenile}$	Study site
							WILD
A_w	Nov_{2006}-Feb_{2007}	Lactation	4	4	1	0	Berenty
B_w	Nov_{2006}-Feb_{2007}	Lactation	4	6	2	1	Berenty
C_w	May-Jul_{2008}	Pregnancy	3	6	1	2	Berenty
D_w	Apr-Jun_{2008}	Mating	6	8	1	3	Berenty
E_w	Mar-Apr_{2011}	Premating	5	5	5	2	Berenty
							CAPTIVITY
A_c	Feb-Mar_{1999}	Pregnancy	2	3	0	0	Pistoia
B_c	Apr-May_{1999}	Lactation	2	4	2	0	Pistoia
C_c	Nov_{2003}-Feb_{2005}	Premating, Mating, Lactation, Pregnancy	4	4	0	2	Pistoia

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Data collection

Systematic data collection was preceded by a training period that lasted until the data collected by the two observers (on aggression and affiliation behavioral patterns) matched in 95% of cases [108]. The excellent visibility condition of the Berenty forest allowed us to apply the same protocol to the wild as was used in captivity. For each agonistic encounter we recorded: (1) identity of the two opponents; (2) aggressive behavioral patterns (mainly chase, bite, grab, jump); and (3) submissive/frightened patterns (flee and vocalization). The agonistic interaction was labeled as "decided" when one of the two opponents gave up the fight (by retreating, fleeing or running away) and the winner could be therefore determined with certainty. For a comprehensive ethogram see [109].

After the last aggressive pattern of any given agonistic event, we followed the loser of the interaction (as the focal individual) for a 10 min post-conflict period (PC). Matched control observations (10 minute long MCs) took place during the next possible day at the same time, context (feeding, resting or travelling) and physiological season (lactation, pre-mating, mating, and pregnancy; see details below) as the original PC. MC data were collected only if all these conditions were met. The MC was conducted on the same focal animal, in the absence of agonistic interactions during the 10 min before the beginning of the MC and when the opponents had the opportunity to interact, within a distance of 10 m maximum [110], [111]).

We considered four groups of affinitive behaviors to identify the first conciliatory contact: body contact (body-to-body contact excluding tails, huddle); greeting (naso-nasal, face grooming); grooming (unidirectional, reciprocal or mutual); olfactory contact (sniffing body, sniffing genitals, and skin licking) [109]). Proximity was not considered because it does not necessarily indicate affiliation. We collected a total of 2339 PC-MC (1461 in captivity and 878 in the wild). For both PCs and MCs we recorded: (1) starting time; (2) type of first affinitive interaction; (3) time of first affinitive contact; (4) partner identity.

Operational definitions and data analysis

Reconciliation analysis was carried out at the individual level, taking the recipient of the aggression as the individual of reference. For each animal we determined the number of attracted, dispersed and neutral pairs over all PC-MC pairs. In attracted pairs, affinitive contacts occurred earlier in the PC than in the MC (or they did not occur at all in the MC), whereas in dispersed pairs the affinitive contacts occurred earlier in the MC than in the PC (or they did not occur at all in the PC). In neutral pairs, affinitive contacts occurred during the same minute in the PC and the MC, or no contact occurred in either the PC or the MC [110].

Due to the small sample size and/or deviation from normality (Exact Kolmogorov-Smirnov, $p<0.05$) we used the Exact Wilcoxon signed-ranks test [112], [113] to compare attracted versus dispersed pairs. Attracted and dispersed pairs were measured at the individual level, thus ensuring the independency of data points. The pair-wise comparison between attracted and dispersed pairs allows checking whether reconciliation is present (if the number of attracted pairs is significantly higher than the number of dispersed pairs) or not.

In addition to determining whether reconciliation was present or not, we assessed the individual rates of conciliatory tendencies of individuals. The measure of corrected conciliatory tendency (CCT; [114]) allows evaluating the level of individual reconciliation by considering the attracted minus dispersed pairs divided by the total number of PC-MC pairs. Individual CCTs were used to determine the mean CCT in wild and captive conditions.

To assess the effect of the different factors on individual CCTs (scalar, dependent variable), we ran two sets of General Linear Mixed Model (GLMM). The first GLMM was performed on all the study groups (Table 1). As fixed factors, we considered sex (binomial: male/female), age (binomial: juvenile/adult), rank position (scalar), season (multinomial: 1–4), individuals (nominal), and groups (nominal). Due to the inter-independence of sex and age, and sex and rank (because females outrank males and adults outrank subadults), these three factors were entered as two combined variables (sex*rank and age*rank). In order to attempt to remove possible confounding variables, the second GLMM was performed only on groups C_c for which data collection had covered all seasons (Table 1). We considered the same fixed factors included in the first GLMM except for group ID.

Since CCT distribution was normal in both cases (Kolmogorov-Smirnov, p = n.s.), an identity link function was used. We tested models for each combination involving the variables of interest, spanning from the null model (only intercept) to the model including all the fixed factors (full model). To select the best model, we used the Akaike's Corrected Information Criterion (AICc), a measure for comparing mixed models based on the −2 (Restricted) log likelihood. The AICc corrects the Akaike's Information Criterion (AIC) for small sample sizes. As the sample size increases, the AICc converges to AIC. The model with a lower value of AICc was considered to be the best model. To avoid the increase of type II errors, factors were excluded from a model only if this improved the model fit by >2 AICc units [115]. The value of degrees of freedom is given by the effective sample size (N) minus the rank design matrix of fixed effects (X). The denominator degree of freedom is estimated by SPSS via Satterthwaite's approximation.

We used all dyadic decided agonistic interactions to prepare a winner/loser socio-matrix and carry out hierarchical rank order analysis, by using MatMan 1.0 based on I&SI rankings (Noldus Information Technology, Wageningen, Netherlands; [116]). To assign the age class to each animal, the individuals were distinguished between adults (regularly performing genital marking, informing an age >18 months) and juveniles (not performing genital marking) [117].

Four seasons were recognized: lactation (1), pre-mating (2), mating (3), pregnancy (4) (The numbers correspond to how the seasons have been entered in the GLMM model). For the captive groups (in the northern hemisphere) the different seasons were: lactating season (group B_c: April-May 1999; group C_c: April-August 2004); pre-mating (group C_c: September-October 2004), mating (group C_c: November-December 2003; November-December 2004), pregnancy (group A_c: February-March 1999; group C_c: January-March 2004; January-February 2005). Individual CCTs for group C_c (observed for more than one season) were calculated using the PC-MC collected for each season. In the wild the mating period varied depending on the group (refer to Table 1 for the groups): pre-mating (group E_w: March-April: 2011), mating (group D_w: April-May-beginning of June 2008), pregnancy (group C_w: May-July 2008), and lactating season (groups A_w and B_w: November-February 2006). The mating period began when at least one female of the group started showing genital swelling from about 1.5–3 cm in length and developing a pink center [57], [58]. In a group, the pregnancy was considered as starting after the last copulation day (confirmed ex-post by births) whereas lactation started when a female in the group gave birth. Overall two mating periods were available in captivity and one in the wild.

Results

A previous study [65] showed that reconciliation was present in captive group A_c but not in group B_c (Table 1) so those analyses are not reported here. The overall CCT calculated here for the first time for all groups was 10.25% ±2.24 (Mean ±SE). In the wild the CCT was 10.99% ±2.44 and in captivity 9.62% ±3.60 (Mean ±SE). Mean CCT% (±SE) for each group are reported in Table 2.

Table 2. Mean Corrected Conciliatory Tendency (CCT %) ± Standard Error (SE) for each study group.

Group	CCT%: Mean±SE
A_w	19.55±7.52
B_w	18.62±8.51
C_w	14.63±6.96
D_w	5.74±2.72
E_w	3.69±2.20
A_c	43.17±19.24
B_c	-14.83±4.23
C_c	9.47±6.73

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For captive group C (Table 1) we found a significant difference between the number of attracted pairs (in which affinitive contacts occurred earlier in the PC than in the MC or they did not occur at all in the MC) and the number of dispersed pairs (in which affinitive contacts occurred earlier in the MC than in the PC or they did not occur at all in the PC; attracted>dispersed pairs: T = 5, N = 10, ties = 1, p = 0.004; Fig 1). In the wild, reconciliation was present in two groups out of five (groups C_w and E_w). In fact, we found a significant difference between attracted and dispersed pairs (attracted>dispersed) for group C_w (T = 0, N = 12, ties = 6, p = 0.031; Fig 2A) and group E_w (T = 2.50, N = 15, ties = 6, p = 0.020; Fig 2B). No significant difference between attracted and dispersed pairs was found for group A_w (T = 0, N = 8, ties = 4, p = 0.125), group B_w (T = 12, N = 11, ties = 2, p = 0.254) and group D_w (T = 19.50, N = 18, ties = 7, p = 0.254).

For both captive and wild settings, the aggression distribution according to the different sex class combination is reported in Table 3 and shows that aggression levels of females toward males and between males were maximum during the mating season. During pregnancy and lactation the majority of conflicts involved females.

Of all the GLMM models tested on all groups (AICc range = 393.675–1107.725) the best one was the full model (Intercept: F = 1.104, df1 = 77, df2 = 38, p = 0.376), including the combination of individual features (sex*rank: F = 1.448, df1 = 1, df2 = 38, p = 0.236; age*rank: F = 0.849, df1 = 1, df2 = 38, p = 0.363), the group identification (F = 1.779, df1 = 1, df2 = 38, p = 0.190), individual identity (F = 0.698, df1 = 64, df2 = 38, p = 0.899), and the season (lactation, pre-mating, mating, and pregnancy; F = 5.282, df1 = 3, df2 = 40, p = 0.004). Fig 3 shows the model output for the best model. Even if part of variability is influenced by individual CCT levels, only the season had a significant effect on the distribution of CCTs, lowest during the mating season (Figs 3 and 4).

Of all the GLMM models tested for group C_c (AICc range = 393.675–534.649), the best one was the full model (Intercept: F = 3.103, df1 = 15, df2 = 38, p = 0.002), including the combination of individual features (sex*rank: F = 1.448, df1 = 1, df2 = 38, p = 0.236; age*rank: F = 0.849, df1 = 1, df2 = 38, p = 0.363), individual identity (F = 1.805, df1 = 9, df2 = 38, p = 0.099), and the season (lactation, pre-mating, mating, and pregnancy; F = 3.844, df1 = 3, df2 = 38, p = 0.017). Fig 5 shows the output for the best model. Again, two individuals accounted for part of the CCT variation but only the season had a significant effect on the distribution of CCTs throughout the year, with CCT values being minimum during the mating season (Figs 5 and 6). Both in captivity and in the wild, males (M_{in}) and females (F_{in}) initiated the first affinitive contact with comparable frequencies in all seasons (captivity, range: M_{in} = 47,22–51.72%; F_{in} = 48.28–52.77%; wild, range: M_{in} = 46,88–50.00%; F_{in} = 50,00–60,00%).

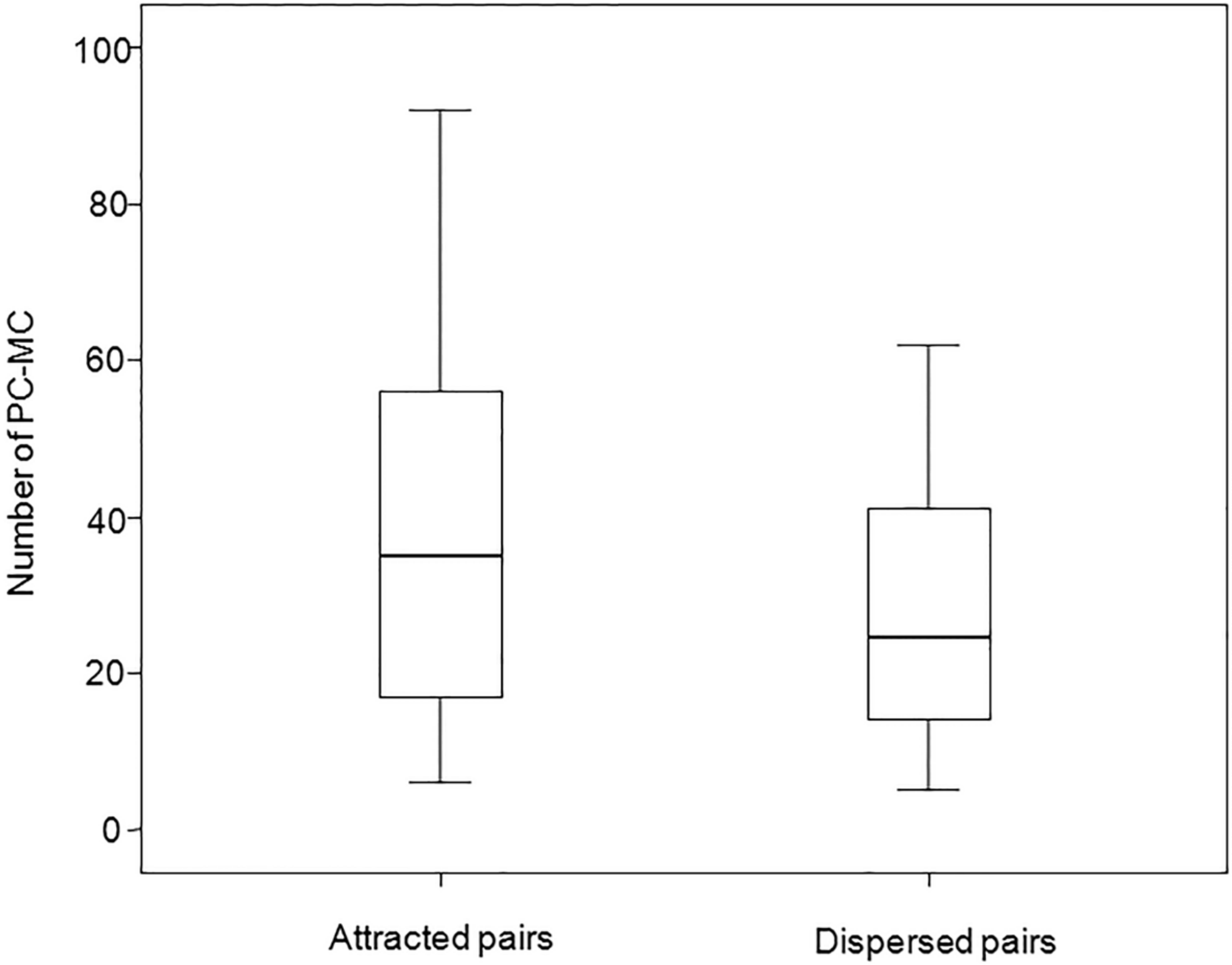

Fig 1. Box plot showing the significant difference (Exact Wilcoxon's test, $p<0.05$) between the number of attracted versus dispersed pairs in the *Lemur catta* troop C_c (November 2003-February 2005), observed at the Pistoia Zoo (Italy). Solid horizontal lines indicate medians; the length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values.

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Discussion

Reconciliation was present both in the wild and in captivity (prediction 1 supported), and specifically in two out of five wild troops of *L. catta* (Fig 2) and in two captive troops (group C_c, present study; group A_c, [65]) (Fig 1). When considering either all the study groups or group C_c only (for which longitudinal data were available), season was the only effect that significantly influenced the fluctuation in the frequency of reconciliation events (Figs 3 and 5). In particular, the conciliatory tendency was lowest during the mating season (prediction 2 supported; Figs 4 and 6).

Reconciliation was found in another despotic species with linear hierarchy, the wolf (*Canis lupus*; mean conciliatory tendency, 44.1% in the wild [11]; 53.3% in captivity [5]). In wolves, each group defends its own territory as a unit [118]. Yet, even if the alpha male normally

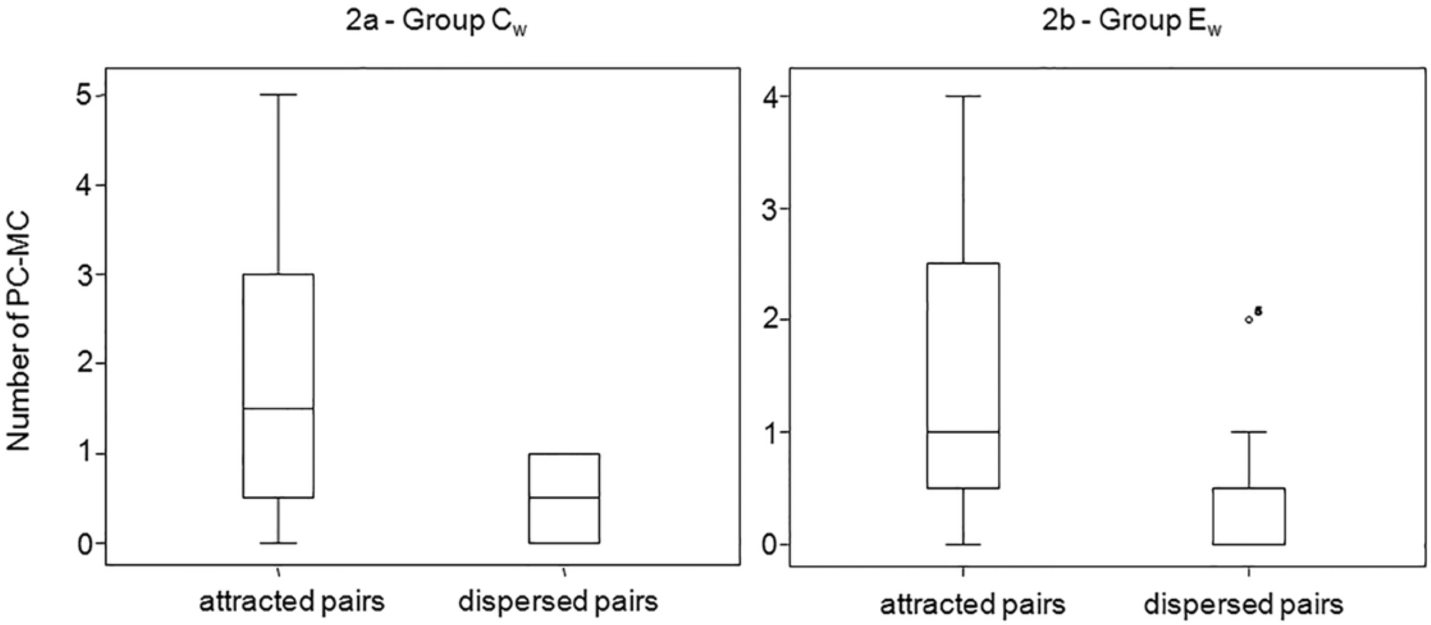

Fig 2. Box plot showing the significant difference (Exact Wilcoxon's test, $p<0.05$) between the number of attracted versus dispersed pairs in two wild *Lemur catta* troops (C_w: May-July 2008, Fig 2a on the left; E_w: March-April 2011, Fig 2b on the right) observed in the Berenty Forest (Madagascar). Solid horizontal lines indicate medians; the length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate range of observed values.

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guides the movements of the wolf pack and initiates aggressions against intruders [118], the subordinate members can sometimes oppose their leader's actions. According to Zimen [119], no subject decides alone the carrying out of activities that are vital to the group cohesion. In short, wolves are highly despotic but also extremely cooperative. The existence of an extremely cooperative pack has presumably to do not only with hunting but also with the collective rearing of offspring and, consequently, with reproductive success [120]. Probably, in wolves the benefit of preserving the social bonds through reconciliation outweighs the cost of pack disruption, which would be detrimental for both dominants and subordinates. Thus, reconciliation can be found in despotic groups provided that they show some form of cooperation [51]. Further evidence of this assumption is the presence of reconciliation in spotted hyenas (*Crocuta crocuta* [53]). Hyenas are despotic but often depend on the help from other group members

Table 3. Aggression distribution (%) according to the different sex class combinations for all seasons, in the wild (W) and in captivity (C). Sex class combinations are: ff (females attacking female), fm (female attacking male), mf (male attacking female), mm (male attacking male).

	ff%	fm%	mf%	mm%
matingC	11,76	56,62	3,68	27,94
prematingC	50	25	12,5	12,5
pregnancyC	43,67	38,61	5,7	12,03
lactationC	51,78	27,74	3,12	17,6
matingW	8,93	65,57	1,1	24,41
prematingW	28,55	56,62	0,12	14,7
pregnancyW	35,21	40,37	0	23,83
lactationW	45,64	41,9	8,23	4,24

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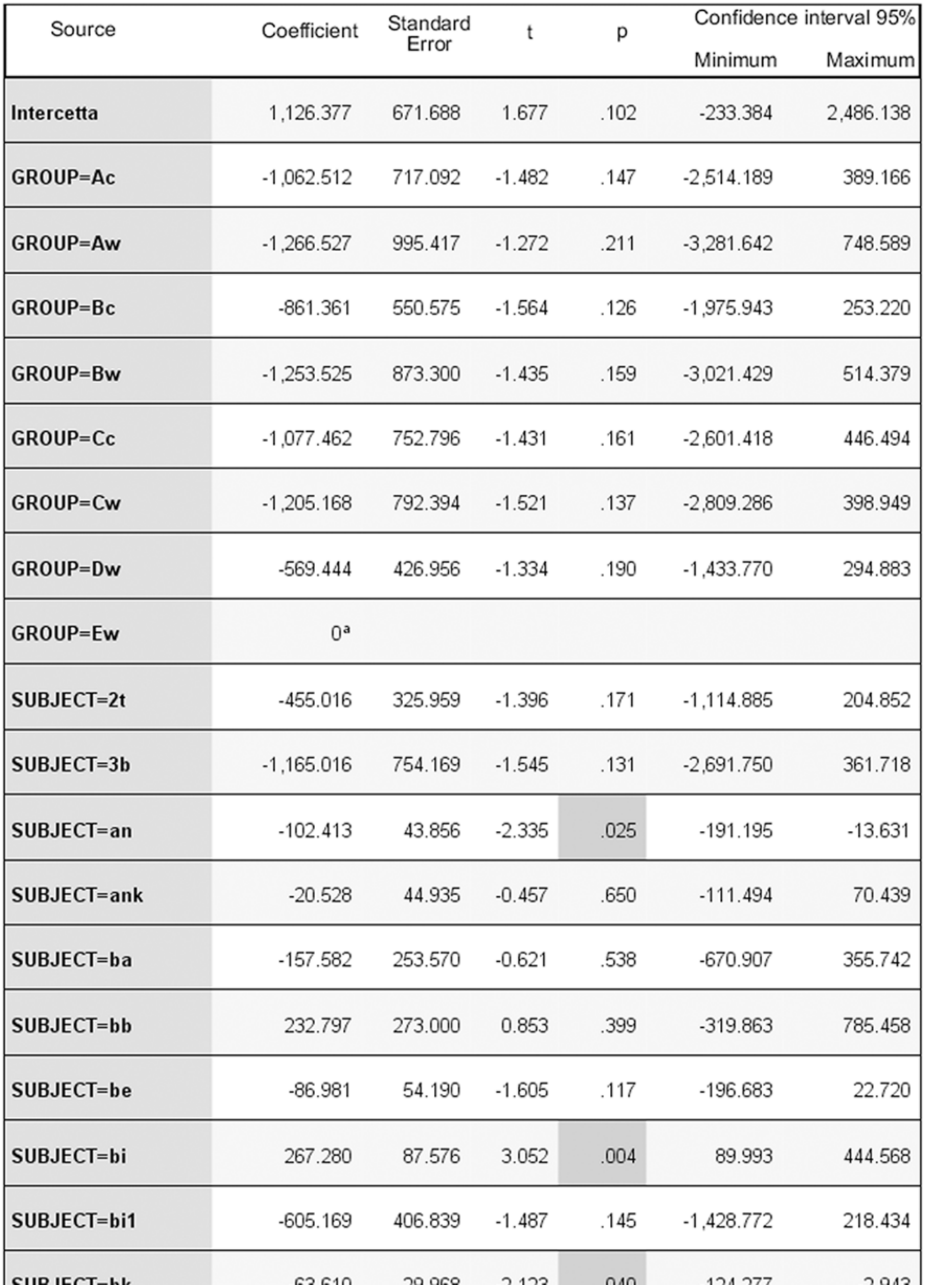

Source	Coefficient	Standard Error	t	p	Confidence interval 95%	
					Minimum	Maximum
Intercetta	1,126.377	671.688	1.677	.102	-233.384	2,486.138
GROUP=Ac	-1,062.512	717.092	-1.482	.147	-2,514.189	389.166
GROUP=Aw	-1,266.527	995.417	-1.272	.211	-3,281.642	748.589
GROUP=Bc	-861.361	550.575	-1.564	.126	-1,975.943	253.220
GROUP=Bw	-1,253.525	873.300	-1.435	.159	-3,021.429	514.379
GROUP=Cc	-1,077.462	752.796	-1.431	.161	-2,601.418	446.494
GROUP=Cw	-1,205.168	792.394	-1.521	.137	-2,809.286	398.949
GROUP=Dw	-569.444	426.956	-1.334	.190	-1,433.770	294.883
GROUP=Ew	0^a					
SUBJECT=2t	-455.016	325.959	-1.396	.171	-1,114.885	204.852
SUBJECT=3b	-1,165.016	754.169	-1.545	.131	-2,691.750	361.718
SUBJECT=an	-102.413	43.856	-2.335	.025	-191.195	-13.631
SUBJECT=ank	-20.528	44.935	-0.457	.650	-111.494	70.439
SUBJECT=ba	-157.582	253.570	-0.621	.538	-670.907	355.742
SUBJECT=bb	232.797	273.000	0.853	.399	-319.863	785.458
SUBJECT=be	-86.981	54.190	-1.605	.117	-196.683	22.720
SUBJECT=bi	267.280	87.576	3.052	.004	89.993	444.568
SUBJECT=bi1	-605.169	406.839	-1.487	.145	-1,428.772	218.434
SUBJECT=bk	63.610	29.968	2.123	.040	124.277	2.943

Fig 3. Output of the best model explaining the distribution of Corrected Conciliatory Tendencies (CCT %, scalar target variable) for all the study groups. AICc = 430, 295. Season: 1 = lactation; 2 = pre-mating; 3 = mating; 4 = pregnancy. Sex: 0 = male; 1 = female. Age class: 0 = subadult; 1 = adult. Rank range: 1–16 (rank position is relative to each group). [a]Redundant coefficients. Please refer to S1 Fig in the Supporting Information for a full size version of Fig 3.

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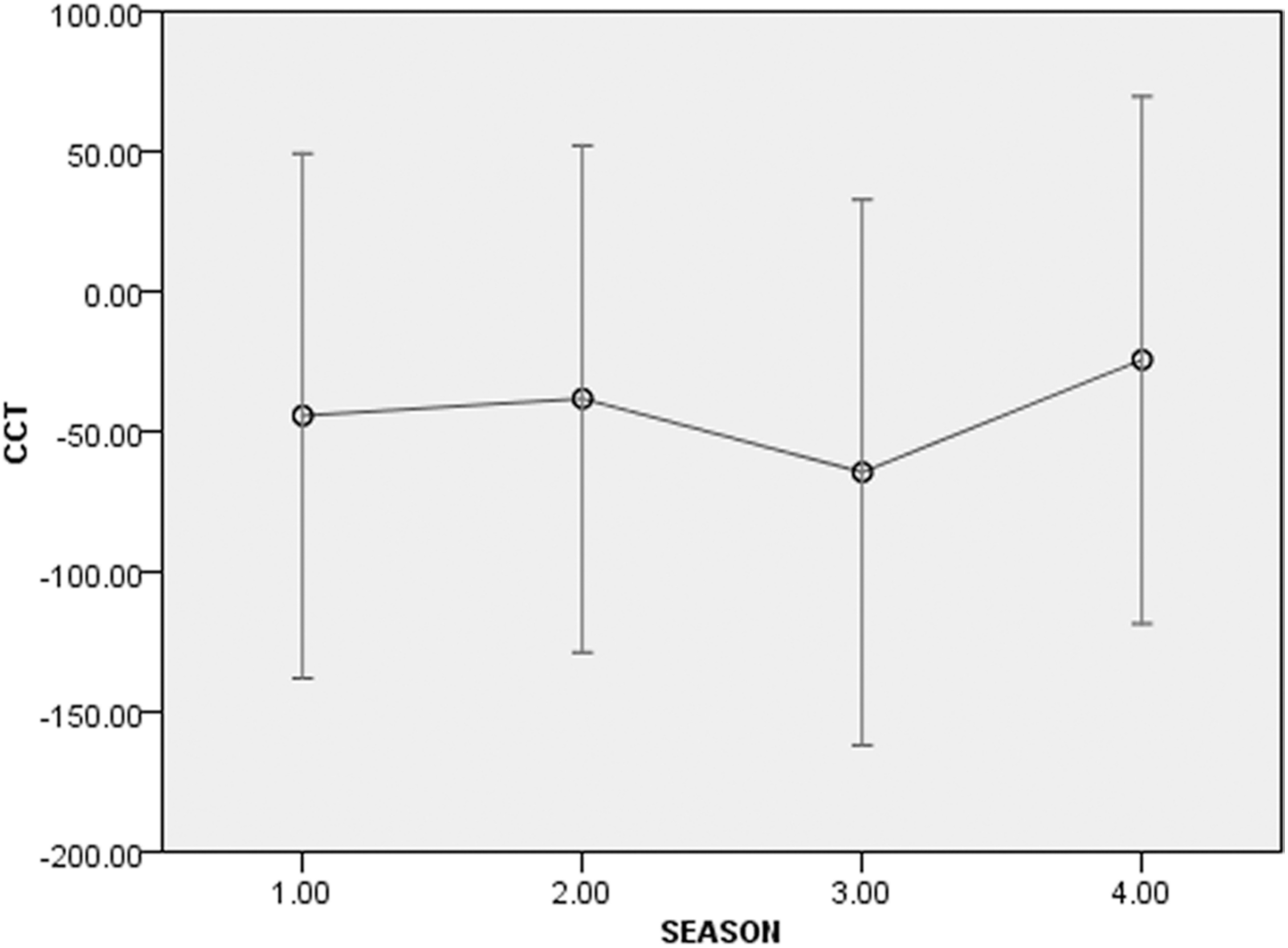

Fig 4. SPSS 20.0 output bar graph showing estimated means of Corrected Conciliatory Tendency (CCT, %) for the significant effect (season: 1 = lactation; 2: pre-mating; 3 = mating; 4 = pregnancy), for all the study groups. Season is the only factor that significantly influences the CCT distribution in the study groups (GLMM; F = 0.718, df1 = 73, df2 = 40, p = 0.890). The conciliatory tendency % is lowest during mating.

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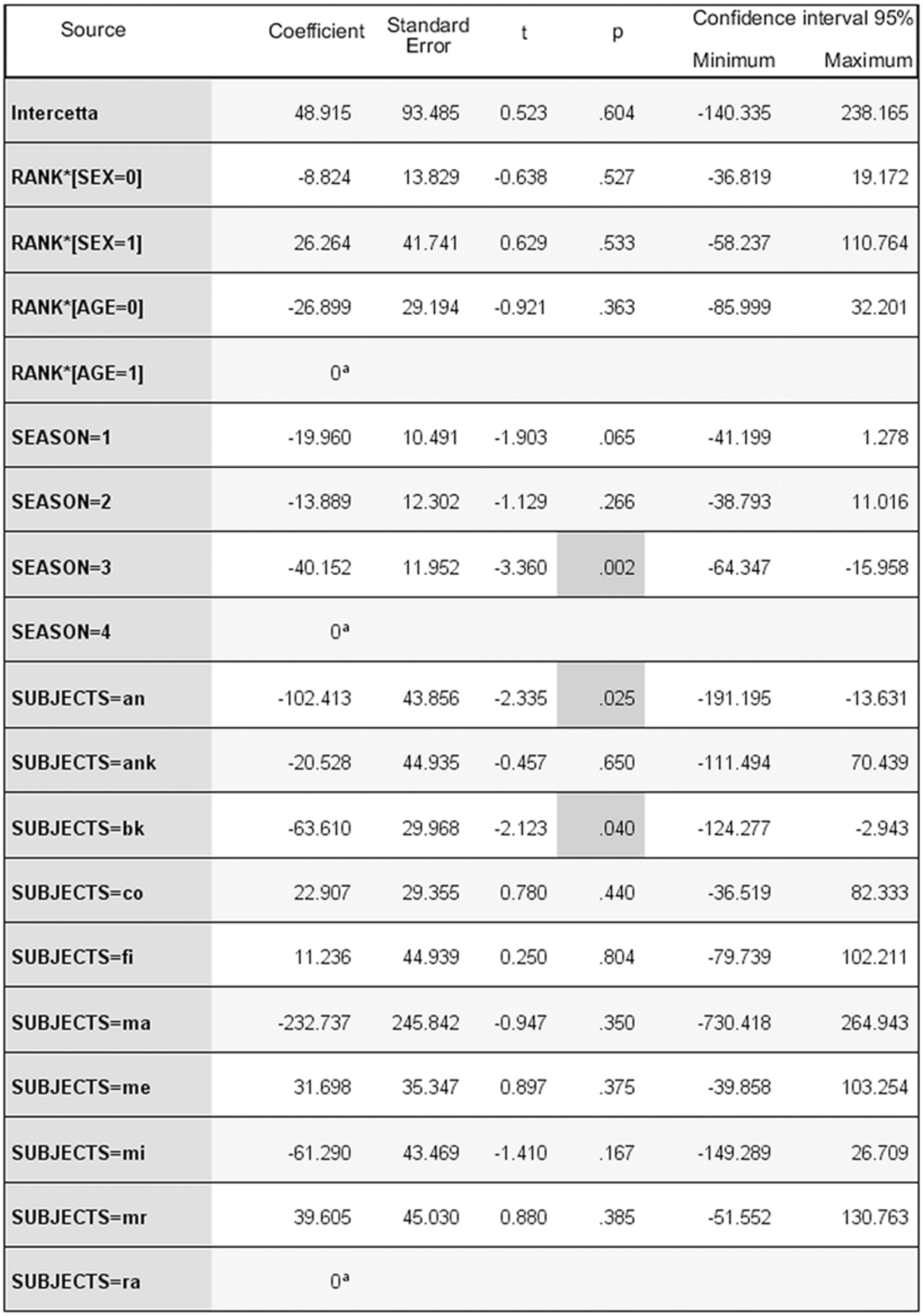
during hunts, defence of ungulate carcasses against competitors, and coalition formation that is important in both the acquisition and maintenance of social rank [53]. Cooperation and despotism are two opposite forces that contribute in shaping reconciliation patterns, as it becomes especially clear when comparing species differing only in some aspects of the social system. In hyenas, as in wolves, the necessity to cooperate overcomes the competition between dominants and subordinates, which explains the presence of reconciliation. The lower levels of reconciliation observed in hyenas (mean conciliatory tendency: 11.3% [53]) may be due to the fact that, contrary to wolves, spotted hyenas live in a fission fusion society allowing dispersal (other than reconciliation) as an exit strategy. The influence of the cooperation pressure over the suitability of engaging in reconciliation is even more evident when comparing spotted hyenas with ring-tailed lemurs. Although both species possess steep female dominance, they strongly differ in the level of cooperation. Unlike hyenas, cooperation in *L. catta* is limited to the coalitionary support provided to the dominant female by other females during targeted aggression toward



Source	Coefficient	Standard Error	t	p	Confidence interval 95%	
					Minimum	Maximum
Intercetta	48.915	93.485	0.523	.604	-140.335	238.165
RANK*[SEX=0]	-8.824	13.829	-0.638	.527	-36.819	19.172
RANK*[SEX=1]	26.264	41.741	0.629	.533	-58.237	110.764
RANK*[AGE=0]	-26.899	29.194	-0.921	.363	-85.999	32.201
RANK*[AGE=1]	0[a]					
SEASON=1	-19.960	10.491	-1.903	.065	-41.199	1.278
SEASON=2	-13.889	12.302	-1.129	.266	-38.793	11.016
SEASON=3	-40.152	11.952	-3.360	.002	-64.347	-15.958
SEASON=4	0[a]					
SUBJECTS=an	-102.413	43.856	-2.335	.025	-191.195	-13.631
SUBJECTS=ank	-20.528	44.935	-0.457	.650	-111.494	70.439
SUBJECTS=bk	-63.610	29.968	-2.123	.040	-124.277	-2.943
SUBJECTS=co	22.907	29.355	0.780	.440	-36.519	82.333
SUBJECTS=fi	11.236	44.939	0.250	.804	-79.739	102.211
SUBJECTS=ma	-232.737	245.842	-0.947	.350	-730.418	264.943
SUBJECTS=me	31.698	35.347	0.897	.375	-39.858	103.254
SUBJECTS=mi	-61.290	43.469	-1.410	.167	-149.289	26.709
SUBJECTS=mr	39.605	45.030	0.880	.385	-51.552	130.763
SUBJECTS=ra	0[a]					

Fig 5. Output of the best model explaining the distribution of Corrected Conciliatory Tendencies (CCT %, scalar target variable) for group C_c. AICc = 398.767. Season: 1 = lactation; 2 = pre-mating; 3 = mating; 4 = pregnancy. Sex: 0 = male; 1 = female. Age class: 0 = subadult; 1 = adult. [a]Redundant coefficients.

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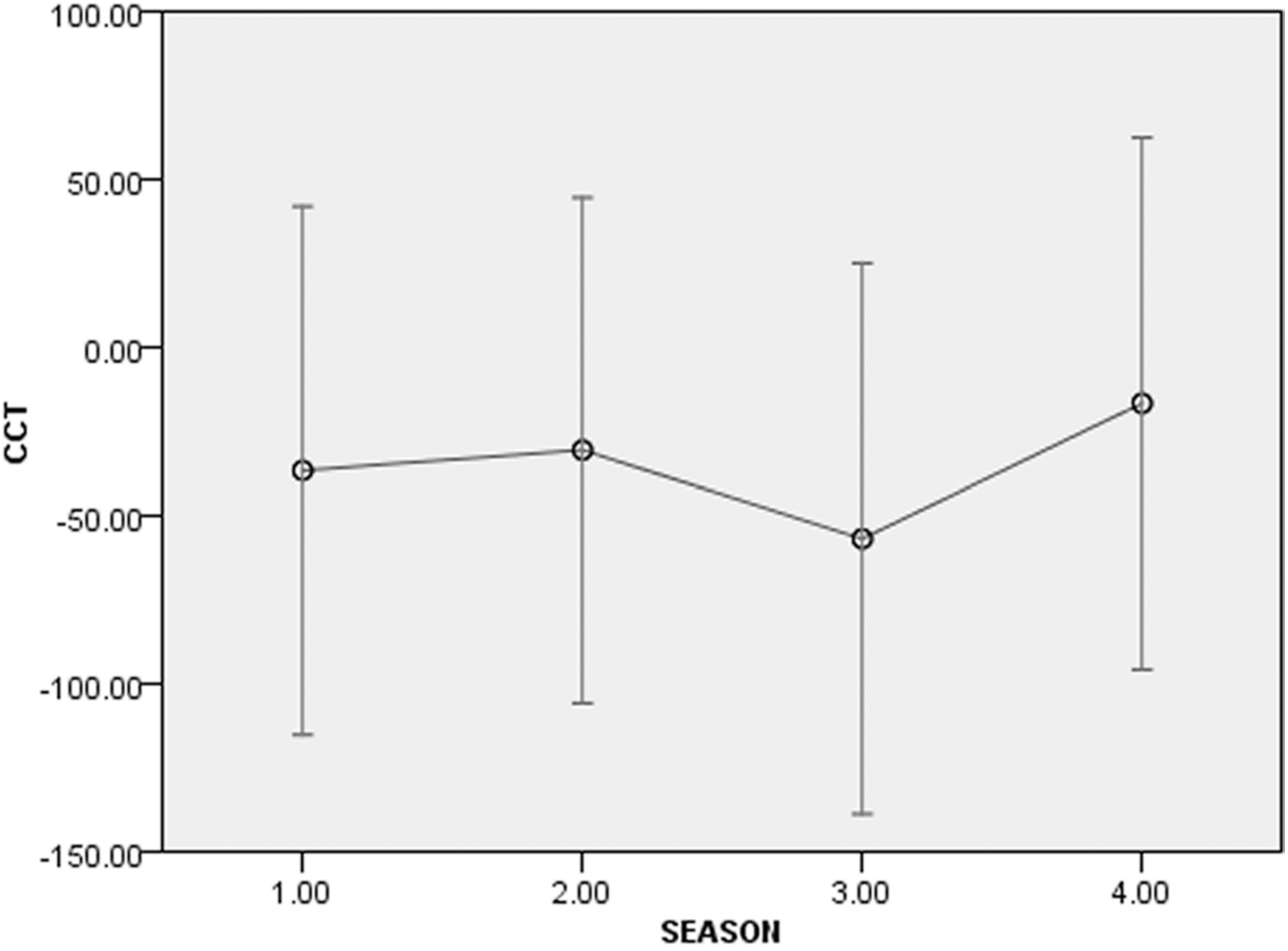

Fig 6. SPSS 20.0 output bar graph showing estimated means of Corrected Conciliatory Tendency (CCT, %) for the significant effect (season: 1 = lactation; 2: pre-mating; 3 = mating; 4 = pregnancy), for group C_c. Season is the only factor that significantly influences the CCT distribution in the study groups (GLMM; F = 1.674, df1 = 15, df2 = 36, p = 0.102). The conciliatory tendency % is lowest during mating.

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conspecifics (to defend territory boundaries or to evict them from the group or the core area of the home range; [10], [58], [121]). This limited cooperation can explain why *L. catta* show the lowest conciliatory tendencies (9–10%). In some macaque species, it has been observed that the higher the cooperation and tolerance levels, the higher the reconciliation rates [52]. This principle can be extended to include other primates. For example, conciliatory tendencies can reach more than 40% in bonobos (*Pan paniscus*) and crested macaques (*Macaca nigra*) [22], [122] and plummet to less than 15% in more despotic and less cooperative species such as Assamese macaques (*Macaca assamensis*) and western gorillas (*Gorilla beringei*) [25], [123]. Of course, the distinction between more and less cooperative species is not always clear cut because primates can form rather complex societies and the individuals of certain subgroups can be more cooperative than the group as a whole, as occurs when cooperative breeding, matriline support, or brotherhood coalitions are in place [124].

Although at low levels, reconciliation seems to be possible in despotic species like *L. catta* when the cooperation-competition balance tilts in favor of cooperation because the benefits of peace making overcome the costs of leaving conflicts unmanaged. But when reproduction is at stake, as it is in lemurs during the once-a-year mating period, both male-female and male-male competition is too high [58],[125] for conflicts to be peacefully resolved. In our study we found that aggression in the mating period was particularly high between males and between females and males (with females initiating the aggression). Consistently, conciliatory rates of both males and females were minimal in the mating season (Figs 4 and 6) likely because in this period the behaviors of individuals are oriented toward reproduction more than maintenance of social stability. Based on these results, it is possible to assert that reconciliation is season-dependent in *L. catta*. Sex was not the explaining variable for the observed fluctuations in conciliatory tendencies. Consistently, both males and females initiated the post-conflict reunion with comparable frequencies throughout the year.

The only study to date that has investigated the seasonal fluctuations of reconciliation in another despotic primate species [84] reported that in female Japanese macaques (*Macaca fuscata*) mating—and not other factors such as changes in activity budgets and dietary composition—had profound effects on peace-making. In fact, the conciliatory tendency–informing reconciliation rates—was significantly lower during the mating season than the non mating season [84]. The authors commented that the negative effects of the mating season on reconciliation within female Japanese macaques may be due to the relevance of female competition for access to male partners in multimale, multifemale societies characterized by adult male dominance. In *L. catta* the situation is reversed: adult females are dominant over males [57–59] and the competition and stress levels during the mating period are highest among males trying to gain access to female partners [125]. Despite the difference in the dominant sex between *L. catta* and *M. fuscata*, the result is similar: reconciliation is lowest during the extremely competitive mating period.

A possible explanation for the seasonal distribution of reconciliation can lie in how hormones modulate the propensity to affiliate with others, and consequently to reconcile. It is worth remembering that the very definition of reconciliation implies the use of affinitive contacts for the purpose of peace making [20]. As well as in other animals in which the sexual context is associated with aggression and competition [126], [127], *L. catta* males experience the highest levels of testosterone during the extremely high competitive mating period [128], which also coincides with the lowest levels of inter-male affiliation [91]. The stress hormones may also increase as a result of aggression, eliciting the fight or flight response [129] and therefore leaving little space for post-conflict affiliation among males. However, literature reports contrasting results on the level of stress hormones (fecal glucocorticoid) in *L. catta* males during the mating period [125].

Besides male affiliation, the high levels of estradiol associated with the mating period can reduce affiliation between primate females, for example in rhesus monkeys (*Macaca mulatta* [130]). Additionally, in human and non human primates, other hormones such as oxytocin and prolactin may influence female affiliation levels throughout the year because they can enhance individual propensity to affiliate and are higher in non-mating periods [131–138]. Consistently, *L. catta* females (aggressors) mainly initiated conciliatory affiliation in group A_c [65]. Therefore, hormonal influence may partly explain the variation in post-conflict conciliatory affiliation across the year.

The seasonality of the conciliatory tendency in *L. catta* documented in the present study is also consistent with the variation of inter-male affiliation rates recorded by Gabriel, Gould & Kelley [91] in the same species, at four sites of Madagascar. These authors observed that inter-male affiliation levels varied across reproductive periods, with the lowest frequencies occurring during the mating period. Overall, the seasonal fluctuations of the reconciliation tendency observed in *L. catta* appear to be sustained by both physiological and socio-ecological data.

Access to females is not the only item worth competing for. Another valuable resource connected to reproductive success is offspring. We observed that in both the wild and captivity female-female aggression was highest during pregnancy and during the lactation period (Table 3), when the newborn is still carried out by the mother. It has been hypothesised that dominant females may target subordinate ones to prevent them from conceiving or to cause them to lose their infants because subordinate females with vital offspring can potentially acquire a higher ranking status in the social group and subtract resources [58], [121]. Food also represents a valuable commodity for the members of social groups, eliciting competition more than cooperation. Consistently, in the wild, reconciliation was found in a group of *Eulemur rufus x collaris* and in two groups of *Propithecus verreauxi* but never in the feeding context [45], [73]. This situation reinforces the idea that when a valuable resource is concerned and cooperation is low (e.g. mate for reproduction, high energy food), gaining access to that resource can be more rewarding than repairing the relationship with a former opponent in the short term, via post-conflict reunions. As a future direction, it would be interesting to assess if and how conciliatory tendencies of *L. catta* are influenced by the context and the individuals involved in the conflicts within each season. We expect that post conflict reunions are lowest in competive contexts (e.g. feeding) and between competing individuals (e.g. females during pregnancy and lactation; males during mating, etc.).

In conclusion, we posit that the ability to reconcile is expressed whenever the benefits of intra-group cooperation overcome the costs of competition, as occurs when a limited, wanted resource is at stake. In summary, this study shows that in despotic social groups in which coalitions are observed, the right question is not *if* but *when* reconciliation can be present.

Supporting Information

S1 Dataset. Dataset used to investigate the occurrence and seasonality reconciliation in *Lemur catta*.
(XLSX)

S1 Fig. Full size version of Fig 3.
(TIF)

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Author Contributions

Conceived and designed the experiments: EP IN. Performed the experiments: EP IN. Analyzed the data: EP IN. Contributed reagents/materials/analysis tools: EP IN. Wrote the paper: EP IN.

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La Sociomatrice Rechargée : de la hiérarchie au profil de dominance dans le lémuriens en liberté

Introduction

La hiérarchie de dominance influence la qualité de vie des animaux sociaux, et sa définition devrait, en principe, être basé sur le résultat d'interactions agonistes. Cependant, la définition et la comparaison des profils de dominance des groupes sociaux est difficile à cause des différentes mesures de dominance utilisées et parce que n'existe pas une mesure qui explique tout. Nous avons appliqué différentes méthodes analytiques à sociomatrices basé sur les conflits gagnés et perdus par les opposants pour déterminer le profil de dominance de cinq groupes de lémuriens sympatriques dans la forêt de Berenty, Madagascar (espèces: *Lemur catta*, *Propithecus verreauxi* et *Eulemur rufus x collaris*). Ces lémuriens sont un excellent modèle d'étude parce qu'elles partagent le même habitat et un profil de dominance apparemment similaire caractérisé par une hiérarchie linéaire et la dominance féminine.

Résultats et Discussion

Les données ont été recueillies pendant plus de 1200 heures d'observation. Notre méthodologie comprenait quatre étapes: (1) en appliquant la méthode de la dominance binaire dyadique (I & SI) soit sur les sociomatrices d'agression que sur celles de évitement nous avons vérifié si la hiérarchie était basé sur l'agression explicite ou, plutôt, sur des comportement de soumission; (2) en calculant les scores normalisés de David (*Normalised David's Scores*) et en calculant la *steepness* (radeur) à partir des sociomatrices d'agression nous avons évalué si la hiérarchie était plus ou moins raide; (3) en comparant les ordres de rang des individus obtenus avec les méthodes 1 et 2, nous avons évalué si hiérarchie était plus o moins invariante; et (4) en évaluant la transitivité triangulaire (*triangle transitivity*) comparée avec l'indice de linéarité et le niveau de la cohésion du groupe, nous avons déterminé si la hiérarchie était plus ou moins cohérent.

Nos résultats montrent que les groupes de *L. catta* ont une hiérarchie cohérente, raide, très transitive et cohésive. Les groupes de *P. verreauxi* sont caractérisés par une hiérarchie modérément raide et cohérente, avec des niveaux variables de transitivité et de cohésion. Le groupe de *E. rufus x collaris* possède une hiérarchie peu raide et incohérent, avec des niveaux plus bais (mais pas minimales) de transitivité et de cohésion. Cette étude démontre que une approche analytique multiple sur les sociomatrices conduit non seulement à une description plus détaillée du profil de dominance, mais permet aussi une comparaisons appropriée entre groupes et espèces différentes.

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The socio-matrix reloaded: from hierarchy to dominance profile in wild lemurs

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ABSTRACT

Dominance hierarchy influences the life quality of social animals, and its definition should in principle be based on the outcome of agonistic interactions. However, defining and comparing the dominance profile of social groups is difficult due to the different dominance measures used and because no one measure explains it all. We applied different analytical methods to winner-loser sociomatrices to determine the dominance profile of five groups of wild lemurs (species: *Lemur catta*, *Propithecus verreauxi*, and *Eulemur rufus* x *collaris*) from the Berenty forest (Madagascar). They are an excellent study model because they share the same habitat and an apparently similar dominance profile: linear hierarchy and female dominance. Data were collected over more than 1200 h of observation. Our approach included four steps: (1) by applying the binary dyadic dominance relationship method (I&SI) on either aggressions or supplant sociomatrices we verified whether hierarchy was aggression or submission based; (2) by calculating normalized David's scores and measuring steepness from aggression sociomatrices we evaluated whether hierarchy was shallow or steep; (3) by comparing the ranking orders obtained with methods 1 and 2 we assessed whether hierarchy was consistent or not; and (4) by assessing triangle transitivity and comparing it with the linearity index and the level of group cohesion we determined if hierarchy was more or less cohesive. Our results show that *L. catta* groups have got a steep, consistent, highly transitive and cohesive hierarchy. *P. verreauxi* groups are characterized by a moderately steep and consistent hierarchy, with variable levels of triangle transitivity and cohesion. *E. rufus* x *collaris* group possesses a shallow and inconsistent hierarchy, with lower (but not lowest) levels of transitivity and cohesion. A multiple analytical approach on winner-loser sociomatrices other than leading to an in-depth description of the dominance profile, allows intergroup and cross-species comparisons.

Subjects Animal Behavior, Anthropology, Zoology
Keywords Lemurs, Sifaka, Steepness, Linearity, Strepsirhines, Prosimians, Madagascar, Social management, Transitivity

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INTRODUCTION

In social animals, an individual's status in its dominance hierarchy can dramatically influence its life quality, including general health, stress levels, resource access, and

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reproductive potential (*Preuschoft & van Schaik, 2000*; *Sapolsky, 2005*). From a social perspective, dominance rank scaffolds the quality of inter-individual relationships and permeates all behavioral spheres (including aggression, affiliation, parental care, and sexual activity) (*Clutton-Brock, Albon & Guinness, 1984*; *Ogola Onyango et al., 2008*; *Palagi, Chiarugi & Cordoni, 2008*; *Norscia, Antonacci & Palagi, 2009*). From an ecological perspective, the structure of dominance relationships can influence reproductive success (*Pusey, Williams & Goodall, 1997*; *von Holst et al., 2002*), resource access (*Clutton-Brock, 1982*; *Krebs & Davies, 1987*), territory quality (*Fox, Rose & Myers, 1981*), predation risk (*Hall & Fedigan, 1997*), and energy budgets (*Isbell & Young, 1993*; *Koenig, 2000*).

Drews (1993) pointed out that the definitions of dominance could be based on theoretical constructs or on certain observable behaviors, and focus on different parameters, such as dyads or single individuals, physical properties of subjects or their role, aggressive encounters or the lack of them. Indeed, the definition of dominance has been based on the confrontation of individuals in agonistic interactions (e.g., *Bernstein, 1981*; *Leiva & de Vries, 2011*) and on other correlates, depending on species-specific behavioral repertoire (e.g., direction of approach-retreats, priority of access, special position, and genital display; *de Waal & Luttrell, 1985*; *Cheney, 1977*; *Kitchen, Cheney & Seyfarth, 2005*; *Parr et al., 1997*; *Murray, 2007*; *Frank, 1986*; *Hirsch, 2010*; *Lemel & Wallin, 1993*; *Alvarez, 1975*).

Within social groups, hierarchies can be either linear ($A > B > C > D$) or non linear (e.g., triangular: $A > B$ and $B > C$ but $C > A$, pyramidal: $A > [B = C = D]$, or class system based: $[A + B] > [C = D + E + F]$). Such feature derives from relational properties of networks of dyads rather than from properties of individuals or single dyads (*Preuschoft & van Schaik, 2000*). In particular, linear hierarchy derives from a set of binary dominance relationships and depends on the number of established relationships and on the degree to which they are transitive (*Landau, 1951*; *Kendall, 1962*; *Appleby, 1983*; *de Vries, 1995*). The degree of linearity can be measured via the corrected Landau's index (h'; *Landau, 1951*), which has been used to determine the structure of dominance relationships in social groups and make comparisons (*Palagi, Antonacci & Norscia, 2008*; *Paoli & Palagi, 2008*; *Hewitt, Macdonald & Dugdale, 2009*). However, hierarchies sharing similar levels of linearity (h') can differ in the extent of power asymmetry between individuals (*Flack & de Waal, 2004*). For this reason *de Vries, Stevens & Vervaecke (2006)* introduced the concept of steepness, another property of dominance hierarchy. The steepness derives from the size of the absolute differences between adjacently ranked individuals in their overall success in winning dominance encounters. When these differences are large the hierarchy is steep; when they are small the hierarchy is shallow. While linearity (based on the binary dyadic dominance relationships) derives from the direction of power asymmetry, steepness requires a cardinal rank measure and considers the extent of power asymmetry (*Flack & de Waal, 2004*; *de Vries, Stevens & Vervaecke, 2006*). However, as pointed out by *de Vries, Stevens & Vervaecke (2006)*, the comparison of the hierarchical structure of different groups using the steepness values has a limitation related to the presence of dyads for which zero interactions were recorded. As it has been shown by *Klass & Cords (2011)* using both simulated and empirical data from four wild monkey groups, the steepness measure is negatively influenced by the proportion of zero dyads in the matrix. If the zero dyads accurately reflect the absence of clear dominance-subordination relationships among individuals, interpreting lower steepness as an indication of less despotic hierarchy is correct. On the contrary, when these zero dyads are due to observational problems, this interpretation is questionable (*de Vries, Stevens & Vervaecke, 2006*).

To avoid the problems related to zero dyads, *Shizuka & McDonald (2012)* and *Shizuka & McDonald (2014)* presented a new measure—based on network structural properties—for determining the level of hierarchy transitivity, less sensitive to observational zeros. This measure, called triangle transitivity (t_{tri}) is based on the transitivity of dominance relations among sets of three individuals that all interact with each other. Triangle transitivity and linearity are essentially equivalent when the dominance relations of all dyads are known but—as discussed above—such conditions are not always met (*Shizuka & McDonald, 2012*). The method by *Shizuka & McDonald (2012)* follows a logic similar to that of *de Vries (1995)*, but the procedure is conducted without filling in zero dyads with randomized dominance relations. In fact, filling in zero dyads artificially decreases the level of linearity because it creates cyclic (and not transitive) triads, e.g., A dominates B, B dominates C, and C dominates A ($A > B > C > A$). According to the framework presented above, it is clear that different aspects of dominance hierarchy can be distinguished that rely on different parameters, thus providing different outcomes. For the first time, we systematically combine different measures into a stepwise approach in order to verify how and whether they add to a more comprehensive definition of the dominance profile of social groups.

As a model for our investigation, we used five wild groups of three sympatric strepsirhine species (*Lemur catta*, *Propithecus verreauxi*, and *Eulemur rufus* x *collaris*) which share the same habitat and part of their home range in the Berenty forest (south Madagascar) and show similar social system features. In fact, they are characterized by multimale-multifemale group composition, linear hierarchy, and exclusive female dominance over males (*Norscia & Palagi, 2011*; *Palagi & Norscia, 2011*; *Sclafani et al., 2012*; *Palagi, Antonacci & Norscia, 2008*). Below, we describe the four steps of the methodological procedure applied in this study. For each step, we formulate predictions on lemurs aimed at assessing whether our approach is able to unveil differences in the dominance profile of social groups whose social system seems alike.

Step 1: In primates, either avoidance or aggression have been used to determine the dominance hierarchy (*Watts, 1994*; *Pruetz & Isbell, 2000*; *Radespiel & Zimmermann, 2001*; *Cooper & Bernstein, 2008*). By running the same test on both avoidance and aggression sociomatrices, based on I&SI rank orders (*de Vries, 1998*), this step allows one to detect if hierarchy linearity is established also via submission patterns other than via overt aggressions.

Lemur catta groups are matrilines with strict dominance hierarchies and are characterized by the presence of formalized subordination vocalizations (*Jolly, 1966*; *Kappeler, 1999*; *Pereira, 2006*; *Koyama et al., 2001*). *Propithecus verreauxi* possesses subordination signals (e.g., submissive chatters) but also a linear hierarchy based on aggression (*Kappeler, 1999*; *Lewis & van Schaik, 2007*; *Palagi, Antonacci & Norscia, 2008*). In this species, aggression by subordinate males toward the dominant males often occur simultaneously with submissive signals (*Lewis & van Schaik, 2007*). *Eulemur fulvus* seems not to possess formalized subordination signals (*Kappeler, 1999*; *Eulemur fulvus* subspecies have been later accorded species status, including *E. rufus* and *E. collaris*; *Mittermeier et al., 2008*). *E. rufus* x *collaris* in Berenty can have a linear hierarchy based on aggressions (*Palagi & Norscia, 2011*). In the light of this framework, we expect that *L. catta* and *P. verreauxi* groups, but not the group of *E. rufus* x *collaris*, may establish a linear hierarchy also using submissive behaviors (avoidance, in this study) (Prediction 1).

Step 2: By using a cardinal rank measure (based on normalized David's scores, see methods) and considering the extent of power asymmetry between individuals (*Flack & de Waal, 2004*; *de Vries, Stevens & Vervaecke, 2006*), this step allows the evaluation of hierarchy steepness of social groups.

Dominance steepness was qualitatively defined as despotic for *L. catta*, egalitarian for *P. verreauxi* and unclear for *E. fulvus* spp. (*Kappeler, 1999*). Therefore, the groups of *P. verreauxi* and *E. rufus* x *collaris* might show less steep hierarchies compared to *L. catta* groups (Prediction 2).

Step 3: Although different, linearity and steepness both rely on the outcome of aggressive encounters between group members (*de Vries, Netto & Hanegraaf, 1993*; *de Vries, Stevens & Vervaecke, 2006*). By comparing the hierarchy obtained via binary dyadic relationships and via normalized David's scores (the two analytical tools used for determining linearity and steepness), this step allows one to detect if the hierarchy remains consistent between methods.

We expect to find higher consistency in *Lemur catta* than in other groups because—based on the information provided at steps 1 and 2—*L. catta* groups normally have a strict hierarchy established via submissive signals and aggression (Prediction 3).

Step 4: By comparing triangle transitivity (*Shizuka & McDonald, 2012*) with the linearity measures, we evaluate the impact that non interacting dyads have on different aspects of dominance hierarchy. By associating this information with the measure of group cohesion around the dominant, we assess whether dominance hierarchy was more or less "cohesive"; that is, more or less influenced by individuals' spatial dispersal.

L. catta and P. *verreauxi* form compact groups, defined as "troops" (*Jolly, 1966*) and "foraging units" (*Richard, 1985*), respectively. Instead, in *Eulemur* spp both males and females show low cohesion levels (*Kappeler, 1999*). Thus, we expect individuals' dispersal to affect hierarchy transitivity more in *Eulemur rufus* x *collaris* than in the groups of the other study species (Prediction 4).

METHODS

Ethics statement

Because the study was purely observational the Animal Care and Use board (University of Pisa) waives the need for a permit. The study was conducted with no manipulation of animals. The study was carried out in the private Berenty Reserve (South Madagascar). The owners Mr De Heaulme (and family) permitted us to conduct the observational study.

Table 1 Data collection information. Table listing group composition, observation period, and time of focal observations.

Study groups	Group composition (age, sex)	Observation period	Observation time
Lemur catta A	6 AF, 3 AM, 1 SAM	March–July 2008	160 hs total; approx. 16 hs/ind
Lemur catta B	6 AF, 5 AM, 2 SAF	March–July 2008	229 hs total; approx. 18 hs/ind
Propithecus verreauxi A	2 AF, 7 AM, 1 SAF	November 2006–February 2007	400 hs total; approx. 40 hs/ind
Propithecus verreauxi B	2 AF, 2 AM, 1 SAM, 1 SAF	November 2006–February 2007	240 hs total; approx. 40 hs/ind
Eulemur rufus x *collaris*	3 AF, 4 AM, 1 SAM, 3 SAF	March–July 2008	177 hs total; approx. 12 hs/ind

Notes.

AF, adult females; AM, adult males; SAF, subadult females; SAM, subadult males.

Study site, groups, and data collection

This study was performed in the Berenty forest (South Madagascar, S 25.00°; E 46.30°). The site is characterized by two main climatic periods: a wet season from October to March and a dry season from April to September (*Jolly et al., 2006*). We observed animals of three sympatric species, and in particular two groups (A and B) of *Lemur catta* (ring-tailed lemurs), two groups (A and B) of *Propithecus verreauxi* (Verreaux's sifaka), and a single group of introduced *Eulemur rufus* x *collaris* (brown lemurs). Group composition is reported in Table 1. In the study we considered both adults and subadults, determined on the basis of scent marking frequency and body size (*Kappeler, 1998*; *Palagi, Gregorace & Borgognini Tarli, 2002*; *Jolly, 1966*).

The physiological seasons (mating, pregnancy, birth and lactating/weaning seasons) influence the frequency of aggressive encounters. In *L. catta*, for example, aggression levels are highest—and conciliatory tendencies lowest—in the period around mating (*Sclafani et al., 2012*; *Palagi & Norscia, 2014*; *Jolly, 1966*). For this reason, observations were conducted in the period around mating for the three species (Table 1). The study groups shared part of their home range. The animals, habituated to human presence, were sexed and individually identified via facial-body features (*Jolly, 1966*).

The observations took place daily from dawn to dusk. The amounts of time devoted to the observations are reported in Table 1. We collected all avoidance submissive behaviors (walk away, cower, flee, and jump away; ethogram: *Pereira & Kappeler, 1997*) via focal animal sampling (*Altmann, 1974*). For submissive behaviors (total: 539 bouts; mean ± SE: 107.80 ± 46,38) we recorded actor's and receiver's identity. We collected data on dyadic agonistic encounters via all occurrence sampling (*Altmann, 1974*), and recorded (i) opponents, (ii) conflict type (decided versus undecided conflicts), and (iii) aggressive patterns (chasing, biting, and slapping). Decided conflicts (total: 957 bouts; mean ± SE: 191.40 ± 64.37) involve a clear winner, with an animal directing an aggressive behavior toward another individual (the victim), which flees or moves away either vocalizing or not. Undecided conflicts involve bidirectional aggressions from an individual to another, with both opponents either moving away or not from the location where aggressive behavior had occurred. Systematic data collection was preceded by training periods that lasted until the observations by the two-three observers matched in 95% of cases (*Martin & Bateson, 1986*). At the end of each training period, Cohen's kappas (k) were higher than 0.70 for all three species (*Kaufman & Rosenthal, 2009*). For each behavioural category (submissive acts and aggressive events) we provide the kappa range (min–max) for all observer dyads: $k_{\text{submissive}} = 0.71\text{–}0.75$; $k_{\text{aggression}} = 0.79\text{–}0.83$. Group size and compositions, and observation periods and time are reported in Table 1.

Each day two observers randomly checked for the level of group cohesion (3–4 times a day) by recording the inter-individual spatial distance (more or less than 20 m) between group-members. *A posteriori* (after determining animals' rank), we calculated the cohesion level at any given time as the number of individuals within 20 m from the dominant female over the total animal number of group members.

Hierarchy linearity, steepness, triangle transitivity and statistical approach

Hierarchy linearity was determined using Matman 1.0 (10.000 randomizations) by determining value of the Landau's corrected linearity index h' (which takes the number of unknown relationships and ties into account) and its statistical significance (*de Vries, Netto & Hanegraaf, 1993*; *de Vries, 1995*). When significant linearity was detected, dominance ranks were determined using the I&SI method and re-ordered to minimize inconsistencies and strengths of inconsistencies in dominance relationships (*de Vries, 1998*). The analysis was conducted on either aggression socio-matrices (based on dyadic decided conflicts) or avoidance socio-matrices.

The steepness was calculated from matrices of decided conflicts via Steepness 2.2 (*Leiva & de Vries, 2011*) and refers to the absolute slope of the straight line fitted to the normalized David's scores plotted against the subjects' ranks (*de Vries, Stevens & Vervaecke, 2006*). Normalized David's scores (NDS) 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 animal having an equal chance of winning or losing in every dominance encounter (*de Vries, Stevens & Vervaecke, 2006*). The correction is necessary when, as in the case of our study groups, the interaction numbers greatly differ between dyads. We determined the NDS-based hierarchy by ranking the individuals according to their NDSs.

In order to assess between-group differences in hierarchical steepness we ran a covariance analysis (One Way Ancova; software: SPSS 20.0). We introduced NDSs as dependent variable; group ID as fixed factor; and rank attributed via NDS as covariate.

After entering data into text files (saved with ".dat" extension) we used the One Way Anova via randomization (Resampling Procedures 1.3 by David C. Howell; 10,000 permutations) to compare cohesion levels and the absolute differences of steepness values between adjacently ranked individuals across groups ($k = 5$). As post-hoc tests we applied the randomization test on two independent samples (between-group comparisons). Randomization procedures account for pseudo-replication (*Manly, 1997*) deriving from non-complete independence of data-points (namely when the same individual is included in more than one data bout).

Table 2 Table referring to the presence of linearity and female dominance based on aggression sociomatrices. Landau's corrected index (h'), level of probability, percentage of unknown and one-way relationships, and Directional Consistency Index (DC) are also reported.

Study groups	Female dominance	Linearity	Landau's corrected index	Unknown relationships	One-way relationships	DC
Lemur catta A	yes[a]	yes[a]	$h' = 0.988$ ($p = 0.0001$)	2.22%	75.56%	0.80
Lemur catta B	yes[a]	yes[a]	$h' = 0.686$ ($p = 0.0001$)	20.51%	73.08%	0.95
Propithecus verreauxi A	yes[b]	yes[b]	$h' = 0.570$ ($p = 0.0350$)	35.56%	37.78%	0.78
Propithecus verreauxi B	yes[b]	yes[b]	$h' = 0.886$ ($p = 0.0700$)	26.67%	53.33%	0.91
Eulemur rufus x *collaris*	yes[c]	yes[c]	$h' = 0.509$ ($p = 0.0370$)	30.91%	52.73%	0.67

Notes.

[a] *Sclafani et al., 2012*.

[b] *Palagi, Antonacci & Norscia, 2008*.

[c] *Norscia & Palagi, 2011*.

By applying the correlation test via randomization we evaluated the correlation between the two hierarchies obtained via both binary dyadic dominance relationships (I&SI) (*de Vries, Netto & Hanegraaf, 1993*) and NDS values (*Leiva & de Vries, 2011*).

We calculated the proportion of transitive triangles relative to all triangles (P_t) and the triangle transitivity metric (t_{tri}) using the codes provided in *Shizuka & McDonald* (*2012*; supplementary material; errata corrige: *Shizuka & McDonald, 2014*). The codes to estimate triangle transitivity were applied on aggression sociomatrices using the package 'statnet' (*Hankcock et al., 2003*) in the R programming environment (*R Development Core Team, 2011*). To this purpose, data were entered in csv files.

RESULTS

Table 2 refers to aggression sociomatrices and shows all of the values related to binary dyadic relationships (I&SI), including Landau's corrected index (h'), unknown and one-way relationships (%), and the Directional Consistency Index (DC). Table 3 shows the other results: linearity derived from avoidance sociomatrices (I&SI method) and outcomes from aggression sociomatrices (steepness, triangle transitivity, and consistency between NDS and I&SI hierarchies).

Avoidance-based matrices did not provide linear hierarchies for *Propithecus verreauxi* and *Eulemur rufus* x *collaris* groups. In contrast, the hierarchy of both *Lemur catta* groups remained linear and showed exclusive female dominance when based on avoidance-based matrices (Table 3). Yet in group A the ranking order in the avoidance based hierarchy was the same observed when the individuals were ordered on the basis of aggression sociomatrices (Table 4) whereas in group B nine individuals out of 13 changed their ranking position in the avoidance based hierarchy (compared to the aggression based hierarchy).

The steepness was highest for *Lemur catta* groups and lowest for the group of *Eulemur rufus* x *collaris*, with *Propithecus verreauxi* groups showing intermediate values (Table 3; Fig. 1). The steepness of hierarchies were significantly different across groups (One-way Ancova; results reported in Fig. 2). Also the absolute NDS differences between adjacently ranked individuals significantly differed across groups (One-way Anova via randomization

Table 3 **Different dominance measures.** Summary of values and/or level of probability referring to linearity (presence/absence) based on avoidance interactions (Landau's corrected index, h'); steepness based on aggression sociomatrices; results of the correlation via randomization (coefficient r and probability); triangle transitivity based on aggression sociomatrices (P_t: proportion of transitive triangles over the total; t_{tri}: triangle transitivity metric); and cohesion around the dominant female. Steepness and triangle transitivity values are based on the matrices of aggressive interactions. The correlation via randomization refers to the correlation between hierarchies obtained via I&SI and normalized David's scores (aggression sociomatrices).

Study groups	Linearity (avoidance interactions)	Steepness	Correlation	Triangle transitivity P_t, t_{tri}	Cohesion around the dominant female
LcA	yes ($h' = 0.751$, $p = 0.0012$)	0.776 ($p = 0.0001$)	$r = 0.99$ ($p < 0.001$)	0.960, 0.839	0.8574 ± 0.0235
LcB	yes ($h' = 0.585$, $p = 0.0040$)	0.460 ($p = 0.0001$)	$r = 0.99$ ($p < 0.001$)	0.996, 0.986	0.8036 ± 0.0347
PvA	no ($h' = 0.376$, $p = 0.2650$)	0.278 ($p = 0.0018$)	$r = 0.90$ ($p = 0.001$)	0.840, 0.360	0.7209 ± 0.0202
PvB	no ($h' = 0.628$, $p = 0.2610$)	0.444 ($p = 0.0015$)	$r = 0.89$ ($p = 0.036$)	1.000, 1.000	0.7321 ± 0.0249
Erxc	no ($h' = 0.350$, $p = 0.2520$)	0.258 ($p = 0.0024$)	$r = 0.83$ ($p = 0.003$)	0.896, 0.582	0.5760 ± 0.0452

Table 4 **Comparison of hierarchical orders assessed according to binary diadic dominance relationships (I&SI) and normalized David's scores corrected for chance (NDS).** Hierarchies of the different lemur groups, *Lemur catta* A (LcA) and B (LcB), *Propithecus verreauxi* A (PvA) and B (PvB), and *Eulemur rufus* x *collaris* (E), assessed according to binary diadic dominance relationships (I&SI) and normalized David's scores corrected for chance (NDS). For all groups, the I&SI and NDS hierarchies deriving from aggression sociomatrices is reported. For the two groups of *L. catta*, the hierarchy obtained via I&SI methods from avoidance sociomatrices was linear. It is reported for LcB only, because for LcA the aggression and avoidance based hierarchies coincide. Grey blocks refer to females and white blocks to males. Females ranking under males are all subadult.

$\text{LcA}_{\text{I\&SI}}$	LcA_{NDS}	$\text{LcB}_{\text{I\&SI-agg}}$	$\text{LcB}_{\text{I\&SI-av}}$	LcB_{NDS}	$\text{PvA}_{\text{I\&SI-agg}}$	PvA_{NDS}	$\text{PvB}_{\text{I\&SI-agg}}$	PvB_{DS}	$\text{E}_{\text{I\&SI-agg}}$	E_{NDS}
M	M	MY	MY	MY	P	MT	CA	BA	TS	OB
T2	T2	CS	CV	S	MT	P	BA	CA	BAPA	TS
TV	TV	S	CS	CS	GR	GR	BO	BRA	OB	BAPA
MS	MS	BI	S	BI	TB	UA	BRA	BO	PAL	PEN
T1	T1	CV	BI	BV	SCR	TB	BRO	BRO	PEN	PAL
BR	BR	BV	CSV	CV	UA	SCR	CL	CL	CM	FF
GR	GR	2T	BV	2T	OT	OT			MCN	CM
BO	BALL	CSV	2T	CSV	U	S			SX	ST
BALL	BO	P	PG	P	N	U			ST	SX
R	R	PG	P	PG	S	N			FF	MCN
		CO	CO	CO					FC	FC
		N	N	N						
		C	C	C						

$F = 2.893$, $df = 4$, $n_{\text{LcA}} = 9$, $n_{\text{LcB}} = 12$, $n_{\text{PvA}} = 9$, $n_{\text{PvB}} = 5$, $n_{\text{Er}} = 10$, $p = 0.036$; n indicates the number of inter-individual NDS differences corresponding to $n - 1$ individuals per group). In particular, both groups of *L. catta* had significantly higher NDS differences than the *E. rufus x collaris* group. A group of *L. catta* (A) also exhibited significantly higher NDS differences than both groups of *P. verreauxi*. In the other *L. catta* group (B), inter-individual NDS differences were significantly higher than those recorded for a group of *P. verreauxi* (A) but comparable to those shown by the other *P. verreauxi* group (B). Results of post-hoc randomization tests on two independent samples are shown in Fig. 3.

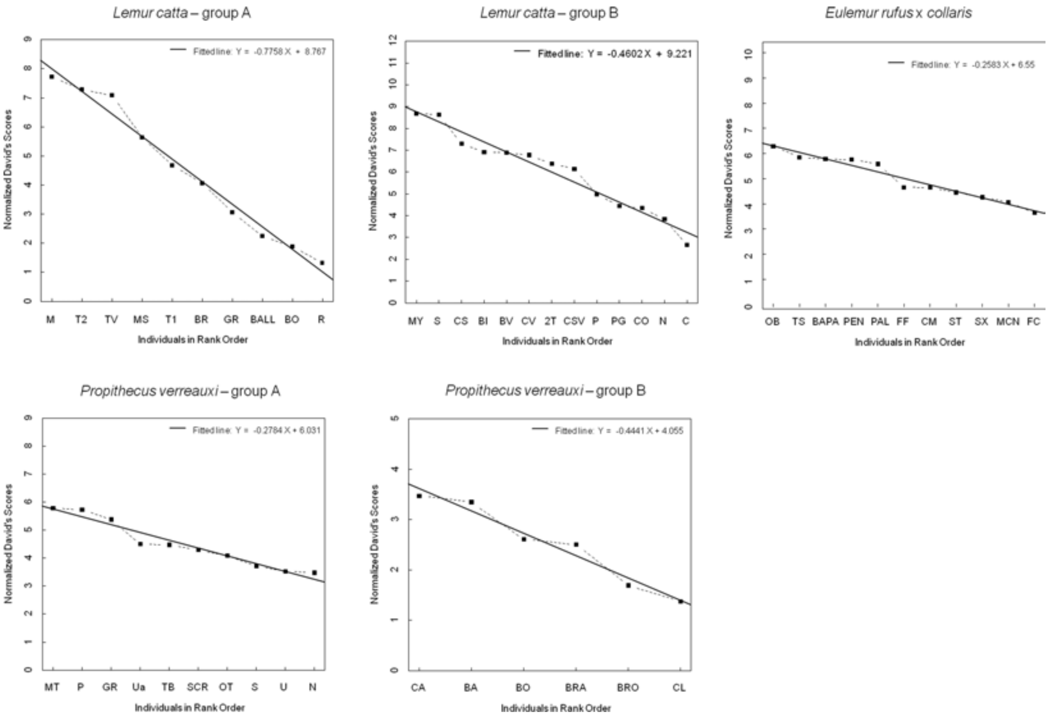
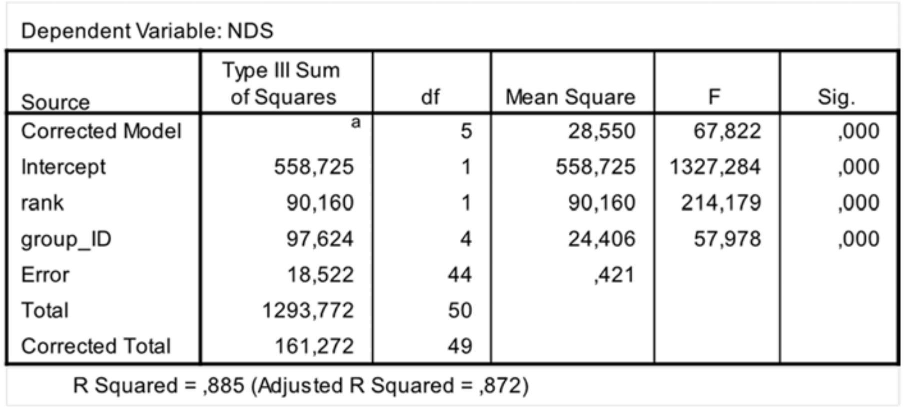


Figure 1 Normalized David's scores plotted against rank order. The graph—output of Steepness 2.2—shows normalized David's scores (corrected for chance, based on aggression sociomatrices) plotted against ordinal rank order (dashed black line), and the fitted line (black, solid line) for all the study groups (*Lemur catta* A and B, *Propithecus verreauxi* A and B, *Eulemur rufus* x *collaris*). The *Y* axis reports the Normalized David's scores and the *X* axis reports the individuals of each group.



Dependent Variable: NDS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	a	5	28,550	67,822	,000
Intercept	558,725	1	558,725	1327,284	,000
rank	90,160	1	90,160	214,179	,000
group_ID	97,624	4	24,406	57,978	,000
Error	18,522	44	,421		
Total	1293,772	50			
Corrected Total	161,272	49			

R Squared = ,885 (Adjusted R Squared = ,872)

Figure 2 Results of the one-way analysis of covariance (ANCOVA). SPSS 20.0 output of the ANCOVA test run to check for between-group differences in hierarchical steepness. Dependent variable: Normalized David's Scores (NDS); Fixed factor: Group ID; Co-variate: rank attributed by NDS.

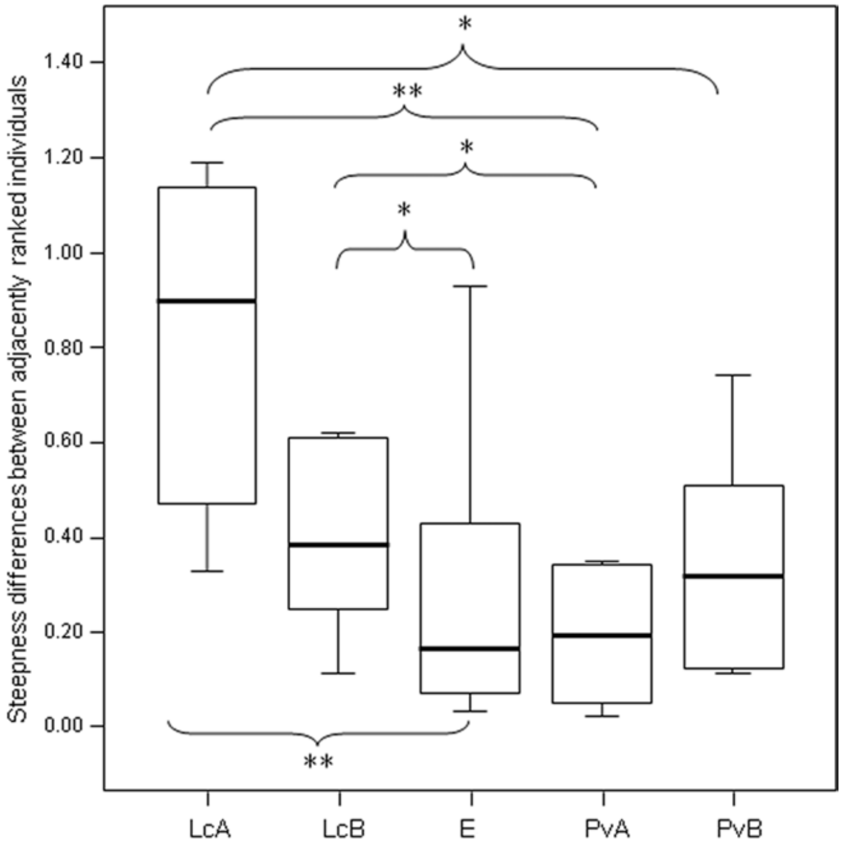


Figure 3 **Difference in the group cohesion around the dominant across the five study groups.** Box plot showing the comparison of the absolute differences of NDS values between adjacently ranked individuals of each group, across the five study groups (LcA, *Lemur catta* A; LcB, *Lemur catta* B; PvA, *Propithecus verreauxi* A; PvB, *Propithecus verreauxi* B; E, *Eulemur rufus* x *collaris*). Sample size (individuals): $n_{\mathrm{LcA}} = 9$, $n_{\mathrm{LcB}} = 11$, $n_{\mathrm{PvA}} = 9$, $n_{\mathrm{PvB}} = 5$, $n_E = 9$. Results of the post-hoc randomization tests on two independent samples: PvB versus PvA: $t = -0.704$, $p = 0.506$; E versus PvB: $t = 0.642$, $p = 0.545$; E versus PvA; $t = -0.068$, $p = 0.943$; PvB versus LcB: $t = 0.160$, $p = 0.281$; PvA versus LcB; $t = 2.150$, $p = 0.046$; PvA versus LcA; $t = 3.479$; $p = 0.005$; PvB versus LcA; $t = 2.225$, $p = 0.044$; E versus LcB: $t = 2.078$, $p = 0.049$; E versus LcA: $t = 3.462$, $p = 0.003$; LcB versus LcA: $t = 0.846$, $p = 0.413$. (*) significant results ($p < 0.05$); (**) highly significant results ($p < 0.01$). Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate the range of observed values.

When—based on aggression sociomatrices—the individuals of each group were ordered according to both I&SI (based on binary dyadic dominance relationships) and their NDS (normalized David's scores) (Table 4), the two resulting hierarchies correlated in all groups. The coefficient indicates that the group of *E. rufus* x *collaris* ($\mathrm{Er}_{\mathrm{I\&SI}}$ versus $\mathrm{Er}_{\mathrm{NDS}}$: $r = 0.83, p = 0.003$) and the two *P. verreauxi* groups ($\mathrm{PvA}_{\mathrm{I\&SI}}$ versus $\mathrm{PvA}_{\mathrm{NDS}}$: $r = 0.90, p = 0.001$; $\mathrm{PvB}_{\mathrm{I\&SI}}$ versus $\mathrm{PvB}_{\mathrm{NDS}}$: $r = 0.89$, $p = 0.036$) had lower correlation levels than the two *L. catta* groups ($\mathrm{LcA}_{\mathrm{I\&SI}}$ versus $\mathrm{LcA}_{\mathrm{NDS}}$: $r = 0.99$, $p < 0.001$; $\mathrm{LcB}_{\mathrm{I\&SI}}$ versus $\mathrm{LcB}_{\mathrm{NDS}}$: $r = 0.99, p < 0.001$), with *E. rufus* x *collaris* showing the lowest correlation coefficient.

Triangle transitivity was highest for group B of *P. verreauxi* ($t_{\mathrm{tri}} = 1$) and for the two groups of *L. catta* ($t_{\mathrm{tri}} = 0.839$; 0.986), and lowest for group A of *P. verreauxi* ($t_{\mathrm{tri}} = 0.360$) and for the group of *E. rufus* x *collaris* ($t_{\mathrm{tri}} = 0.582$) (Table 3).

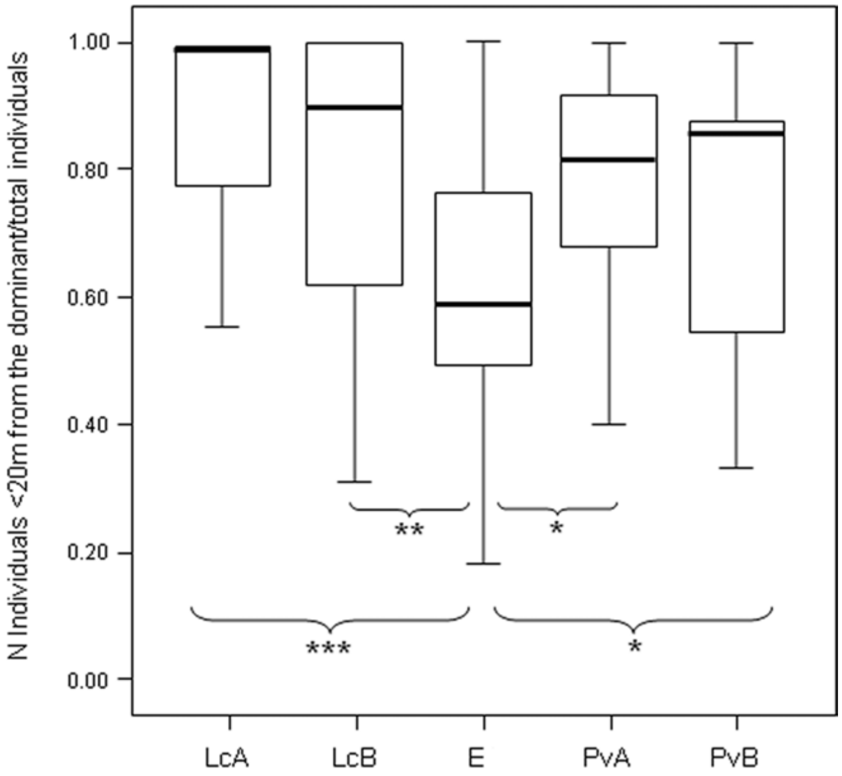


Figure 4 **Difference in the group cohesion around the dominant across the five study groups.** Box plot showing the difference in group cohesion around the dominant (proportion of individuals within 20 m from the dominant over the total animal number) across the five study groups (LcA, *Lemur catta* A; LcB, *Lemur catta* B; PvA, *Propithecus verreauxi* A; PvB, *Propithecus verreauxi* B; E, *Eulemur rufus* x *collaris*). Observational cohesion bouts for the five groups: $n_{\mathrm{LcA}} = 65$, $n_{\mathrm{LcB}} = 40$, $n_{\mathrm{PvA}} = 60$, $n_{\mathrm{PvB}} = 77$, $n_E = 34$. Results of the post-hoc randomization tests on two independent samples: PvB versus PvA: $t = -1.656$, $p = 0.101$; E versus PvB: $t = 2.101$, $p = 0.036$; E versus PvA; $t = 2.355$, $p = 0.021$; PvB versus LcB: $t = -1.800$, $p = 0.080$; PvA versus LcB; $t = -1.592$, $p = 0.121$; PvA versus LcA; $t = -1.581$; $p = 0.118$; PvB versus LcA; $t = -1.901$, $p = 0.058$; E versus LcB: $t = -2.995$, $p = 0.004$; E versus LcA: $t = -3.840$, $p < 0.001$; LcB versus LcA: $t = -0.326$, $p = 0.752$. (*) significant results ($p < 0.05$); (**) highly significant results ($p < 0.01$); (***) extremely significant results ($p < 0.001$). Solid horizontal lines indicate medians; length of the boxes corresponds to inter-quartile range; thin horizontal lines indicate the range of observed values.

After determining the dominant individual based on NDS hierarchy (aggression sociomatrices), we found that the proportion of group members packed around the dominant female at any given time (group cohesion) significantly differed across the five groups (Anova One-Way Randomization $F = 7.173$, $df = 4$, $n_{\mathrm{LcA}} = 65$, $n_{\mathrm{LcB}} = 40$, $n_{\mathrm{PvA}} = 60$, $n_{\mathrm{PvB}} = 77$, $n_{\mathrm{Er}} = 34$, $p < 0.001$; n indicates the observational cohesion bouts). Post-hoc randomization tests on two independent samples revealed that group cohesion significantly differs between the *E. rufus* x *collaris* group and the groups of the other two species (statistical results are shown in Fig. 4).

DISCUSSION

As indicated in previous reports, all the groups under study are characterized by linear hierarchy and female dominance determined using aggression sociomatrices (*Norscia & Palagi, 2011*; *Palagi & Norscia, 2011*; *Sclafani et al., 2012*; *Palagi, Antonacci & Norscia, 2008*). Based on these characteristics only, we would conclude that similar dominance features apply to all groups. The multistep approach proposed here allows the drawing of a more detailed dominance profile of social groups, thus leading to a fine-grained distinction between them.

Aggression- and submission-based hierarchy (step 1, prediction 1)

The first step of our approach allows the detecting of how hierarchy linearity is established (via either overt aggressions or avoidance, or both) in different social groups. We used avoidance, not elicited by any aggressive behavior but indirectly correlated with the outcome of decided agonistic encounters, to verify whether it provides the same dominance structure (linearity, female dominance) obtained via aggression sociomatrices. The two *L. catta* groups stand out because they maintained linearity whereas the other groups did not (Tables 2 and 3; Prediction 1 partly confirmed). Contrary to the prediction, *P. verreauxi* groups did not have a linear hierarchy based on avoidance probably because the use of avoidance behavior does not reflect the use of formalized submissive chatters and/or because the hierarchical relationships are more relaxed (*Kappeler, 1999*; *Norscia, Antonacci & Palagi, 2009*). In *L. catta* groups the linearity of avoidance based hierarchy derives from the highest frequency of unidirectional dyadic avoidance behavior in *L. catta* groups and it can indicate greater acceptance of the inferior social rank to dominants by subordinates (deference), greater intolerance by dominants to subordinates, or both. We define hierarchy here as aggression-based if it is exclusively unveiled by overt aggressions and submission-based if its detection does not necessarily depend on an arena of aggressive encounters. According to this definition, linear hierarchy is both aggression- and submission-based in *L. catta* groups and aggression-based in *P. verreauxi* and *E. rufus* x *collaris* groups.

Previous works have reported the coexistence of more than one hierarchy at the same time, often behavior dependent. *Richard (1974)* in *Propithecus verreauxi* detected no consistent correlation between the rank of individuals ordered according to the criterion of priority of access to food (feeding hierarchy) and their rank established according to the frequency of aggression, the direction and frequency of grooming, or preferential access to females during the mating season. *Alvarez (1975)* observed that hierarchy in squirrel monkeys (*Saimiri sciureus*) varied from quasi-linear to circular, depending on the behavioral patterns considered for rank assessment (approaching, following, withdrawing, and genital inspection). *de Waal & Luttrell (1985)* described behavior dependent hierarchies and distinguished between real and formal dominance relationships in rhesus macaques (*Macaca mulatta*), with the former depending on agonistic encounters and the latter only depending on unidirectional and context independent signals (*de Waal, 1982*; *de Waal, 1986*). Similarly, a troop of ringtailed lemurs (group B) showed behavior dependent hierarchy. In fact, in this group the ranking order obtained via avoidance sociomatrices differed from the ranking order generated by aggression sociomatrices

(Table 4). Even though the same leader and exclusive female dominance were maintained in both aggression and avoidance based hierarchies, many individuals possessed a different position in the two hierarchies (Table 4). Thus, the power discrepancy perceived by individuals (asymmetry derivable from avoidance behavior) does not necessarily go in tandem with the asymmetry established via aggressive interactions.

The difference observed in the ranking order and linearity level is also related to the lower number of avoidance events compared to decided agonistic encounters recorded in the study groups, which is in line with the fact that in the period around mating aggression rates are higher than in other periods in wild lemurs (*L. catta*: *Jolly, 1966*; *Gould & Ziegler, 2007*; *P. verreauxi*: *Brockman, 1998*; *Brockman et al., 1998*; *Eulemur rufus*: *Ostner, Kappeler & Heistermann, 2002*).

The twofold approach presented here, which considers both submissive and aggressive interactions, unravels divergences between perceived and aggression based power asymmetry in species that are classically considered as despotic (e.g., baboons, *Rowell, 1967*; mandrills, *Wickings & Dixson, 1992*; wolves, *Cordoni & Palagi, 2008*).

Shallow versus steep hierarchy (step 2, prediction 2)

The second step allows separating social groups according to hierarchy steepness. When steepness is used to evaluate the dominance structure based on aggression sociomatrices, other inter-group differences—not revealed by linearity—emerge. The different groups indeed differed in their hierarchical steepness (Fig. 1; Fig. 2). The comparison of dyadic NDS values across groups allowed segregating the *L. catta* groups from the group of *E. rufus* x *collaris*, with ringtailed lemur groups showing the steepest hierarchy gradient. Conversely, *P. verreauxi* groups and the *E. rufus* x *collaris* group showed similar steepness levels. Prediction 2 is overall supported but it is worth remarking that the differences in steepness levels between *L. catta* and *P. verreauxi* groups varied depending on the groups considered (Fig. 3). This situation is in line with the observations of *Balasubramaniam et al. (2012)* on different macaque species (ranked from grade 1 to 4 depending on their tolerance levels). The authors observed that steepness measures were more continuous than other measures (e.g., counter-aggression) and did not fully match the species separation into different tolerance grades. Consequently, they noted that different aspects of social style may display somewhat different patterns of variation across species, and that covariation between even closely related measures may be imperfect (*Balasubramaniam et al., 2012*).

Weakly versus strongly consistent hierarchy (step 3, prediction 3)

The third step allows differentiating groups according to another property: hierarchy consistency. By way of both I&SI (binary dyadic dominance relationships) and NDS (normalized David's scores corrected for chance) methods, all adult females outranked adult males in all study groups, thus confirming the exclusive dominance of females over males (Table 4). Overall the I&SI and NDR correlated in all groups and were quite consistent, even if the top ranking female remained the same only in the two ringtailed lemur groups (Table 4). Therefore, the hierarchy appears to be more rigid in *L. catta*, apparently sealing off individual movement within the hierarchy (cf. Tables 2–4; Prediction 3 confirmed).

As specified above, different ranking positions in the same group can be observed for the same individuals when they are context or behavior dependent (e.g., present study, aggression- versus submissive-based hierarchy in *Lemur catta*; *Richard, 1974*; *Alvarez, 1975*; *de Waal & Luttrell, 1985*). In the case of our study groups, the two different hierarchical arrangements, especially detectable in sifaka and brown lemurs (Table 4), are both generated by the same aggression sociomatrices, through the application of different analyses: I&SI which focuses on the direction of aggression asymmetry; and NDS, which also considers the extent of aggression asymmetry and dyadic encounter probability. It is the quantitative approach itself that reveals two different hierarchy properties.

Less versus more cohesive hierarchy (step 4, prediction 4)

The measure of triangle transitivity (excluding dyads without interactions; t_{tri}; *Shizuka & McDonald, 2012*) provides a further (and different) clustering of our study groups, with a group of *P. verreauxi* (B) and the two groups of *Lemur catta* showing the highest transitivity levels, and *E. rufus* x *collaris* and a group of *P. verrauxi* (A) the lowest levels (Table 3). The lower transitivity values observed for *E. rufus* x *collaris* and a group of *P. verrauxi* (A) (compared to the other study groups) correspond to weaker group cohesion around the dominant (Table 3), even if the groups of *L. catta* and *P. verreauxi* did not significantly differ in the cohesion levels (Fig. 4) (Prediction 4 only partially confirmed). On the other hand, the highest levels of triangle transitivity in *L. catta* just confirm the rigid ranking order, corresponding to the highest group packing around the dominant. The top triangle transitivity value was recorded for the group B of *P. verreauxi*. Because the number of known relationships in this group is smallest (Table 2), the likelihood of finding a relatively large t_{tri} value in this group is larger than in the other groups where the numbers of known relationships are much larger. *Shizuka & McDonald (2012)* reported that the proportion of zero dyads is positively correlated with group size and ten out of twelve groups of six individuals included in their study showed maximum triangle transitivity ($t_{\mathrm{tri}} = 1$). The tightest bonds linking group members in *L. catta* and *P. verreauxi* (Fig. 4; Table 3) fit with previous literature, which refers to ring-tailed lemur and sifaka as cohesive units (*Jolly, 1966*; *Richard, 1985*). In a behavioral ecology perspective, the high group dispersion observed in brown lemurs is consonant with their habitat use pattern. At Berenty, they tend to extend resource exploitation in terms of diet variety (*Jolly et al., 2000*; *Pinkus, Smith & Jolly, 2006*), amount of food intake (*Simmen, Hladik & Ramasiarisoa, 2003*), temporal activity (*Donati et al., 2009*) and ranging patterns (*Tanaka, 2007*). The higher is the spatial dispersion of an animal group, the lower is the level of contact opportunities. This can explain, at least in this group, the higher percentage of unknown relationships (Table 2) leading to less transitive relationships. Another possibility is that the observed inter-species variations in dominance property may emerge not just from ecological, but also from phylogenetic constraints. It is not the prerogative of this study to test the explanatory models put forward by sociobiologists that posit variation in dominance relationships (e.g., *Lewis, 2002*; *Hemelrijk, Wantia & Isler, 2008*; *Wilson, 2000*) but future work should attempt to do so.

In our case, it is possible to state that *L. catta* and *P. verreauxi* groups show more cohesive hierarchies than *Eulemur rufus* x *collaris*, meaning that in the two former species a higher proportion of group members is found close to the dominant females (within 20 m) at any given time. However, the level of relationship transitivity is higher in *Eulemur rufus* x *collaris* than in one group of *P. verreauxi* (Table 3). We could interpret this very last result (*Eulemur rufus* x *collaris* not showing the lowest transitivity values of all) as a result biased by the presence of non-interacting diads. In fact, if patterns of non-interactions are not random because some dyads do not actually interact, the formation of transitive versus cyclic triangles can be skewed (*Shizuka & McDonald, 2012*). Instead, we speculate that the comparison between triangle transitivity and linearity provides the hierarchy assessment with an added value because it suggests that in the core group of *Eulemur rufus* x *collaris* (composed by individuals that actually interact with each other) relationships are more transitive than it appears by considering linearity alone. The observation of the different cohesion levels helps in explaining this difference by reinforcing the idea that hierarchy is less cohesive in the brown lemur group because the presence of non interacting dyads (informed by the weak group cohesion around the dominant) does not affect transitivity (non interacting dyads excluded) as much as it affects linearity (non interactive dyads included).

CONCLUSIONS

We applied a four-step approach on a large database gathered, with the same observation protocol, on five wild multimale-multifemale lemur groups. The groups shared the same habitat, and part of the home range, and they were all characterized by linear hierarchy and female dominance (*Norscia & Palagi, 2011*; *Palagi & Norscia, 2011*; *Sclafani et al., 2012*; *Palagi, Antonacci & Norscia, 2008*). This information alone would lead to conclude that their dominance profile is alike. We used different measures (linearity, steepness, consistency, triangle transitivity and group cohesion) to determine whether group hierarchy was (i) aggression or submission based; (ii) shallow or steep; (iii) weakly or strongly consistent; and (iv) more or less cohesive.

Lemur groups showed different types of similarities and dissimilarities depending on the measure used. For example, dominance relationships of the *E. rufus* x *collaris* group and *P. verreauxi* groups are similar according to the steepness levels but can be different according to triangle transitivity and group cohesion. *L. catta* groups are more similar to *P. verreauxi* groups in terms of group cohesion, but not necessarily in terms of triangle transitivity or steepness. *Lemur catta* and *E. rufus* x *collaris* largely differ in steepness and level of linearity. Overall, *L. catta* groups show a linear, steep, consistent and highly transitive and cohesive hierarchy. *P. verreauxi* groups show a linear, moderately steep and consistent hierarchy, with variable levels of triangle transitivity and cohesiveness. *E. rufus* x *collaris* shows a linear but shallow and inconsistent hierarchy, with lower (but not lowest) levels of transitivity and scarce cohesiveness (but more groups should be considered to accurately assess this last property).

In conclusion, the use of the same method (I&SI) applied to different behavioral databases (aggression/avoidance), and different methods (normalised David's scores, binary dyadic dominance relationships, triangle transitivity) applied to the same behavioral database (aggression sociomatrices), resulted in different dominance outlines relative to the same study subjects. The use of different methodological approaches is important because each single measure has its own limits: for example, linearity does not appreciate the different extent of power asymmetry between individuals, steepness can suffer from the presence of zero dyads, triangles of individuals may not be fully independent because each triangle within a social network can share nodes (individuals) and ties (connections) with other triangles (*Flack & de Waal, 2004*; *de Vries, Stevens & Vervaecke, 2006*; *Wasserman & Katherine, 1994*). Finally, a multiple analytical approach can lead to a more in-depth description of dominance profile, which is a multilevel concept combining many aspects of social dominance.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Ivan Norscia and Elisabetta Palagi conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Because the study was purely observational, the Animal Care and Use board (University of Pisa) waives the need for a permit. The study was conducted with no manipulation of animals. The study was carried out in the private Berenty Reserve (South Madagascar). The owners Mr De Heaulme (and family) permitted us to conduct the observational study.

Supplemental Information

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Vérification de différentes hypothèses sur le bâillement dans deux espèces de lémuriens en liberté: *Propithecus verreauxi* et *Lemur catta*

Introduction

Le bâillement, bien que facilement reconnaissable, est difficile à expliquer. Les explications traditionnelles souligne les mécanismes physiologiques subjacents le bâillement, mais plus récemment il y a eu une attention croissante vers les processus comportementaux qui peuvent influencer le bâillement. Cette étude est la première à tester une série d'hypothèses sur le bâillement dans des populations de primates en liberté. Nous avons étudié deux espèces de strepsirrhine sympatriques, le *Lemur catta* et le *Propithecus verreauxi,* qui vivent dans la réserve de Berenty, forêt d'Ankoba (24.99 ° S, 46.29 ° E), située dans le sud de Madagascar. Le dimorphisme sexuel est absente dans les deux espèces. Par contre, leurs différences dans les caractéristiques écologiques et comportementales facilitent des tests comparatifs de différentes hypothèses sur le bâillement.

Résultats et Discussion

Nos résultats montrent que dans chaque espèce les mâles et les femelles bâillaient avec des fréquences similaires, en accord avec l'hypothèse du dimorphisme, qui prédit que un faible dimorphisme sexuel conduit à peu de différences intersexuelles dans le bâillement. À l'appui de l'hypothèse du changement d'état physiologique nous avons observé que les fréquences de bâillement était liée au cycle veille-sommeil et ponctuait les transitions d'un comportement à l'autre. Les fréquences étaient significativement plus élevés chez *L. catta* que chez *P. verreauxi,* parce que *L. catta* a un niveau d'activité de base plus élevé et par conséquent un nombre plus élevé de transitions comportementales. En accord avec l'hypothèse qui voit le bâillement associé avec le niveau d'anxiété, les fréquences de bâillement augmentait considérablement 10 minutes après des attaques prédatrices ou agressions. Nos résultats fournissent la première preuve empirique d'une connexion directe entre l'anxiété et le bâillement chez les lémuriens. Nos résultats montrent que le bâillement dans ces deux strepsirrhines se produit dans des contextes différents, mais d'autres recherches seront nécessaires pour déterminer s' il y a différents types de bâillement et si ces types peuvent être considérés dans le cadre d'un comportement unitaire.

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RESEARCH ARTICLE

Testing Yawning Hypotheses in Wild Populations of Two Strepsirrhine Species: *Propithecus Verreauxi* and *Lemur Catta*

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Yawning, although easily recognized, is difficult to explain. Traditional explanations stressed physiological mechanisms, but more recently, behavioral processes have received increasing attention. This is the first study to test a range of hypotheses on yawning in wild primate populations. We studied two sympatric strepsirrhine species, *Lemur catta*, and *Propithecus verreauxi*, of the Ankoba forest (24.99°S, 46.29°E, Berenty reserve) in southern Madagascar. Sexual dimorphism is lacking in both species. However, their differences in ecological and behavioral characteristics facilitate comparative tests of hypotheses on yawning. Our results show that within each species males and females yawned with similar frequencies supporting the *Dimorphism Hypothesis*, which predicts that low sexual dimorphism leads to little inter-sexual differences in yawning. In support of the *State Changing Hypothesis* yawning frequencies was linked to the sleep-wake cycle and punctuated transitions from one behavior to another. Accordingly, yawning frequencies were significantly higher in *L. catta* than in *P. verreauxi*, because *L. catta* has a higher basal level of activity and consequently a higher number of behavioral transitions. In agreement with the *Anxiety Hypothesis*, yawning increased significantly in the 10 min following predatory attacks or aggression. Our findings provide the first empirical evidence of a direct connection between anxiety and yawning in lemurs. Our results show that yawning in these two strepsirrhines occurs in different contexts, but more research will be necessary to determine if yawns are a single, unitary behavior. Am. J. Primatol.

Key words: ring-tailed lemurs; sifaka; sexual dimorphism; behavioral transitions; anxiety

INTRODUCTION

Yawning has long been a subject of evolutionary biology. Darwin (1872) described yawning as an act of deep inspiration, followed by a lengthy, forceful expiration with simultaneous contraction of many skeletal muscle groups. Yawning can be easily recognized in mammals and even birds [Gallup et al., 2009]. Many authors have offered physiological hypotheses to explain yawning. It has been hypothesized that yawning is modulated by factors such as respiration, circulation, brain oxygenation, thermoregulation, arousal and the sleep-wake cycle [Gallup, 2014; Giganti & Zilli, 2011; Guggisberg et al., 2010; Matikainen & Elo, 2008]. These base-line physiological functions do not rule out the possibility that yawning has social and communicative roles in some taxa [Gallup, 2011]. In primates, Altmann [1967] de] defined three different types of yawns: the "drowsiness yawn" (strongly dependent on the sleep-wake cycle), the "tension yawn" related to anxiety, and the "threat yawn" used to display canines during aggressive encounters. In many primate species characterized by high sexual dimorphism, yawning is often used as an aggressive, threat signal, emitted by high-ranking males [Adams & Shoel, 1982]. This link between sexual dimorphism and male threat yawning is probably related to both intra-group rank competition and inter-group territorial defense [*Macaca fascicularis*, *M. nigra*, *M. fuscata*, *Theropithecus gelada*; Deputte, 1994; Hadidian, 1980; Leone et al., 2014; Troisi et al., 1990]. Sex differences in yawning are less evident in species characterized by low levels of sexual dimorphism, especially in canine size [*Pan paniscus*, Demuru & Palagi, 2012; *Homo sapiens*, Schino & Aureli, 1989; *Pan troglodytes*, Vick & Paukner, 2010].

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Provine [1986, 2005] attempted to combine multiple behavioral state changes associated with yawning (wakefulness to sleep, sleep to wakefulness, alertness to boredom, etc.) within a single framework and stated, "yawning is a vigorous, widespread act that may stir up our physiology and facilitate these transitions". Several reports indicated that yawns serve to stimulate or facilitate arousal during state changes [Baenninger, 1997; Provine, 2005; Vick & Paukner, 2010; Walusinski & Deputte, 2004]. These reports led to the general consensus that yawning, as well as scratching and other self-directed behaviors [Buckley & Semple, 2012; Tinbergen, 1952], anticipates important events and behavioral transitions. In humans [Giganti & Zilli, 2011] and geladas [Leone et al., 2014] spontaneous yawning shows daily fluctuations linked to the sleep-wake cycle. Yawning is probably associated with increasing activity levels even outside the sleep/wake context [Baenninger et al., 1996]. In chimpanzees yawning is related to changes in the level of general activity with increased locomotion during the one-minute interval preceding and following a yawning event. Thus yawning could be related to social synchronization by punctuating changes in behavioral activity [Vick & Paukner, 2010].

Yawning may also be affected by stressful environmental and socially stressful stimuli [Liang et al., 2015; Schino et al., 1990]. Laboratory studies on birds and mammals showed that yawning frequency initially decreases or remains unchanged in the first 20-min following a stressful event. As the effect of the anxiogenic events clears, yawning generally increases in a 20–40 min time window [Miller et al., 2010; Miller et al., 2012; Moyaho & Valencia, 2002]. In primates there are only anecdotal reports on the possible linkage between stressors and "tension yawns." In *Macaca nigra*, for example, low ranking adult males yawned frequently after dominant males had approached and sat nearby [Hadidian, 1980]. When two unfamiliar female macaques were paired in a relatively small cage there was an increase in the frequency of yawning in both subjects perhaps due to stress between individuals for whom there was not yet a clear-cut dominance relationship [Schino et al., 1990]. Wild chimpanzees yawn more frequently in the presence of humans [Goodall, 1968] and captive chimpanzees yawn more in response to social tension [Baker & Aureli, 1997]. Recent studies on chimpanzees [Vick & Paukner, 2010] and geladas [Leone et al., 2014] support the idea that different forms of yawning can have different functions. In particular, the "tension yawn" seems to be linked to anxiety even though yawning has been mostly studied in association with other well-known displacement behaviors (like scratching) and rarely analyzed independently [Pomerantz & Terkel, 2009]. In *L. catta* yawns were observed in contexts of unclear dominance reversals and during intergroup conflicts [Pereira & Kappeler 1997], suggesting that there might be a link between yawning and potentially stressful events. However, the relationship between yawns and disturbing events was never demonstrated. As for many behavioral topics, lemurs have been neglected for the study of the mechanisms underpinning yawning behavior. The lemurs, found exclusively in Madagascar, represent an independent radiation from continental primates [Tattersall, 1982]. Comparing strepsirrhines with the better-known haplorrhines may be useful because these two primate taxa, although distantly related, share a long period of common ancestry in which common foundations of yawning may have been forged. Investigating yawning and testing some of its possible functions in strepsirrhines can add some pieces to the complex picture characterizing the evolution of this puzzling behavior in primates. To test various hypotheses of yawning we studied two sympatric species of strepsirrhines living in multimale-multifemale groups characterized by linear hierarchy, female dominance and male dispersal [Jolly, 1966; Richard, 1974]: *Propithecus verreauxi* (Fig. 1a) and *Lemur catta* (Fig. 1b). We tested three hypotheses, as follows:

1. *The Dimorphism Hypothesis*: Except for some differences in the sexual distribution of scent glands, *L. catta* and *P. verreauxi* show no obvious sexual dimorphism. Males and females have similar body size, coat color, and length of canines [Lewis, 2002; Pereira & Kappeler, 1997]. Because *L. catta* and *P. verreauxi* lack sexual dimorphism, we expect no difference in the frequency of yawns between males and females in either species (Prediction 1).
2. *The State Changing Hypothesis*: If yawning is involved in behavioral transitions, the frequency of yawning should increase with such transitions (from behavior A to behavior B) ($\mathrm{YW}_{\textit{in between}\ \text{behaviors A and B}} > \mathrm{YW}_{\textit{in between}\ \text{behaviors A and A}}$) (Prediction 2a). As yawning is influenced by the sleep-wake cycle, we predicted spontaneous yawning to peak during transition to and from periods of rest (Prediction 2b). *L. catta* is more active than *P. verreauxi*, which spends a large part of the day resting for fiber digestion due to its folivorous diet [Jolly, 1966; Norscia et al., 2006]. Therefore, yawning should be more frequent in *L. catta* than in *P. verreauxi*, because *L. catta* has more frequent transitions between one state and another (Prediction 2c).
3. *The Anxiety Hypothesis*: Yawning seems to be associated to tense situations in primates [Goodall, 1968; Hadidian, 1980; Schino et al., 1990; Baker & Aureli, 1997; Pomerantz & Terkel, 2009; Leone et al., 2014], including lemurs [Pereira & Kappeler, 1997]. If yawning is indeed a behavioral response to anxiety its frequency should increase in both study species after exposure to stressful stimuli (Prediction 3).

Fig. 1. *Propithecus verreauxi* (a) and *Lemur catta* (b) yawning. (Photos by Ivan Norscia & Elisabetta Palagi).

METHODS

Ethics Statement

This study was approved by the University of Pisa (Animal Care and Use board). Since the study was purely observational, without any kind of animal manipulation, the committee waived the need for a permit. The study was carried out in the private Reserve of Berenty (South Madagascar) and De Heaulme family (the owner) permitted us to observe the animals. This research adhered to the American Society of Primatologists Principles for the Ethical Treatment of Primates.

Study species, Location, and Subjects

Lemur catta (ring-tailed lemur) and *Propithecus verreauxi* (Verreaux's sifaka) are two lemur species living in multimale-multifemale groups characterized by linear hierarchy, female dominance and male dispersal [Jolly, 1966; Richard, 1974]. The two study species have overlapping distributions in southern Madagascar and share many ecological, social and behavioral features. However, one notable difference between these species is that *L. catta* is omnivorous while *P. verreauxi* has a highly specialized and energetically poor, folivorous diet [Jolly, 1966; Norscia et al., 2006; Richard, 1974; Warren & Crompton, 1998].

This study was conducted in the gallery forest of Berenty, a 200ha reserve on the Mandrare River in southern Madagascar [for a complete description of the study site see Jolly et al., 2006], specifically in the northern part of the forest called Ankoba (24.99°S, 46.29°E), a 40ha secondary forest 50–60 years old, with canopy at 10–15 m (except for a few emergent acacias to more than 20 m).

We observed one group of *L. catta* and three groups of *P. verreauxi* (A, B and C) with overlapping home ranges. The *L. catta* troop was composed of six adult females, five adult males, two subadult males and three infants. The *P. verreauxi* groups were composed as follows: group A included three adult females, four adult males, one subadult male and one infant; group B included two adult females, three adult males, one subadult male and two infants, and group C comprised one adult female, two adult males and one infant. Infants from both species were not included in the analyses.

Data Collection, Procedures, and Definitions

All subjects were habituated to human presence and individually identified via facial-body features [Jolly, 1966]. Data collection was carried out from March to May 2011. Observations were dictated to a tape recorder and later entered into a computer database. Three individuals (the first author and two field assistants) made observations from 6 a.m. to 6 p.m. daily. Data collection was independent as each observer recorded the behavior of different subgroups of animals.

Prior to the beginning of data collection used in this study the three observers underwent a training period (the trainers were the second and the last author). During the training phase, the same focal animal was simultaneously followed by the three observers, and the data were then compared. The training period lasted about one week and ended when the reliability between observers (N = 3 possible pairs) reached 95% [Martin & Bateson, 1986]. Inter-observer reliability was checked not only during the training phase but also at the beginning of each month of observation by comparing the behavioral bouts collected by the different observers (Cohen's Kappa never below 0.85).

We collected the exact sequence of each behavioral state (resting, moving, feeding, social and autogrooming, sleeping, foraging, self-licking and marking behaviors) and yawning via focal animal sampling [Altmann, 1974]. We also focused on behavioral patterns immediately preceding and following each yawning event. Each focal observation lasted 20 minutes (*L. catta*—individual hour mean 23.93 ± 1.3 SE; *P. verreauxi*—individual hour mean 18.30 ± 0.1 SE). To evaluate and compare levels of behavioral activity of the two species, we extracted data from the focal observations of each individual. We then randomly selected a 3-min time window and counted the number of behavioral transitions performed by the focal animal. For this analysis, we only considered baseline 3-min time blocks (e.g., in absence of perturbing factors that could alter the behavior of the subjects) to ensure that comparison made between data gathered in the same condition.

The presence of three observers concurrently working on the same group and the spatial cohesion of its members allowed us to apply the all occurrences sampling method [Altmann, 1974], often unfeasible in the wild. We thus collected all the yawning events performed by each subject each time the subject was visible (*L. catta* - individual hour mean 108.23 ± 4.49 SE; *P. verreauxi*—individual hour mean 79.70 ± 4.34 SE). We recorded the identity of the yawner, the exact time, the group context (resting, feeding or moving), the presence (social condition) or absence (solitary condition) of at least one conspecific in proximity (less than two meters) to the yawner. Within 1 min after it had yawned, the external body temperature of that animal was recorded via Noncontact Infrared Thermometer Lafayette TRP-19 from a distance of 5–10 m. Three data points per lemur were collected on hairless head zones and the mean ± SE was calculated. In order to quantify the individual hourly frequency of yawns, the number of yawns performed by the subject was normalized on the total of observation hours collected on that subject when he/she was awake. In order to understand if the species with the higher level of behavioral activity showed a higher frequency of yawns, we compared the hourly frequencies of yawning in *L. catta* and *P. verreauxi*. Independent datasets from the three observers were combined into a single file for analyses.

During observations, we recorded potentially disturbing events, which included (i) intra-group aggression involving the focal subject as victim (we collected 406 aggressive encounters for *L. catta* and 53 aggressions for *P. verreauxi*), (ii) presence of potentially dangerous stimuli within 10 m (e.g., tourists, dogs, snakes) eliciting an alarm vocalization in at least one group member [*P. verreauxi*: roaring barks and tchi-faks; Fichtel & Kappeler, 2011; *L. catta*: grunts to barks; Doyle & Martin, 1979; Macedonia, 1990, 1993], iii) predatory attacks by raptors (e.g., *Polyboroides radiatus*) We recorded 47 events for *L. catta* and 50 events for *P. verreauxi*.

Pre-Post Yawning Observation [PPY/MC Method]

In order to understand if yawning marked behavioral transitions, we defined the Pre-Post Yawning condition (PPY) compared to a Match Control observation (MC). This focal methodology was modified from the PC/MC method developed for studying post-conflict behavior in primates [de Waal & Yoshihara, 1983]. In the PPY condition we recorded behavioral transitions, considering only the changes from one behavioral pattern to another one [resting, moving, feeding, foraging, self-grooming, self-licking and marking behaviors; Buckley & Semple, 2012]. Following Vick & Paukner [2010]'s methodology for assessing yawn variation in chimpanzees, we recorded all behaviors occurring within the minute preceding and the minute following each yawn thus defining a 2-min around-yawning observation period ($1_{\text{pre-min}}$ / Y / $1_{\text{post-min}}$). On the next possible day we obtained an MC observation. We followed the same focal individual recording the behaviors occurring within a 2-min time window, at the same hour, in the same context, but in absence of yawning.

Post Distress Observation: the PD/MC Method

We defined a Post Distress period (PD) compared to a Matched Control observation (MC) [de Waal & Yoshihara, 1983]. To collect PD observations on yawning we considered three main disturbing events: intra-group aggressions, presence of unfamiliar stimuli within 10 m from the observed animal, and predatory attacks on the group. We started a PD all occurrences observation on yawning, lasting 60 min on the study subject, if one of the previously described disturbing conditions was satisfied. The few available studies [*Rattus norvegicus*; Moyaho & Valencia, 2002; *Melopsittacus undulatus;* Miller et al., 2010; *Sula granti*, Liang et al., 2015] showed that the yawning response to stressful stimuli does not necessarily increase in the first 10 min but it can increase after 20 and/or 40 min. Therefore, the 1-hour PD observation considered in this study was divided into 3 blocks (0–10 min, 10–20 min, 20–60 min). We gathered a total of 99 hours of PD observation for *L. catta* and 43 hours for *P. verreauxi*.

We performed the MC observations of yawning on the same individual on the next possible day, at the same hour, in the same context but in absence of any perturbing event (baseline level of yawning). Time blocks (as defined above) were also obtained from the MC observations. The number of yawns performed during PD observations in the three time blocks was compared with that emitted in the three time blocks under control condition (MC). To exclude the possibility that yawning was due to a possible synchronization response, we excluded from the analyses the yawns performed by subjects who were able to perceive yawns previously emitted by others in the 10 minutes time-window of the PD and MC conditions.

Statistical Analysis

The analyses were carried out using individuals as test cases (rows). Due to the non-normal distribution of data (Kolmogorov-Smirnov, $P < 0.05$) we employed nonparametric statistics [Siegel & Castellan, 1988]. To examine the influence of sex and species on the distribution of yawning we used the Exact Mann–Whitney test. To compare the hourly-mean frequencies of yawning estimated for each time slot we used the Exact Friedman test. To compare the PPY and MC frequencies of yawn and its frequency in the PD and MC periods we used Exact Wilcoxon signed-rank test. All the tests were two-tailed and the level of significance was set at 5%.

RESULTS

The Dimorphism Hypothesis

The hourly frequency of yawns did not differ between males and females in either species: *Propithecus verreauxi* (Exact Mann-Whitney $U = 26.00$, $N_{\text{males}} = 10$, $N_{\text{females}} = 6$, $P = 0.707$); *Lemur catta* ($U = 15.00$, $N_{\text{males}} = 7$, $N_{\text{females}} = 6$, $P = 0.445$) (Prediction 1 supported).

The State Changing Hypothesis

The hourly frequencies of spontaneous yawning fluctuated significantly during the day in both *Propithecus verreauxi* (Friedman: $\chi^2 = 24.426$, $df = 9$, $N = 16$, $P = 0.004$) and *Lemur catta* (Friedman: $\chi^2 = 39.214$, $df = 9$, $N = 13$; $P = 0.00001$), with a peak observed in the period around resting (12.00-15.00 h for *Propithecus verreaxi*; Fig. 1, and 12.00-14.00h for *Lemur catta;* Fig. 1b) (Prediction 2a). The external body temperatures recorded within 1 min of yawns were not highest during the period of maximum yawning activity (cf. Fig. 2a and 2b, and Table I).

In the solitary context (no conspecifics within 2 m), the frequency of the behavioral transitions preceding (Pre) and following (Post) yawns (PPY) was significantly higher than in the absence of yawns (Matched-Control, MC) in both species (Exact Wilcoxon test: $T_{P.verreauxi} = 51.5$, $ties = 0$, $N = 12$, $P = 0.012$; Fig. 3a; $T_{L.catta} = 45$, $ties = 0$, $N = 11$, $P = 0.004$, Fig. 3b). The frequency of the behavioral transitions preceding (Pre) and following (Post) yawns (PPY) was significantly higher than in the absence of yawns (Matched-Control, MC) also in social context (presence of at least one conspecific within 2 m) ($T_{L.catta} = 76$, ties $= 0$, $N = 13$, $P = 0.001$; $T_{P.verreauxi} = 78$, ties $= 0$, $N = 12$, $P = 0.0001$) (Prediction 2b supported).

The number of behavioral transitions per unit of time was significantly higher in *L. catta* than in *P. verreauxi* (Exact Mann-Whitney: $U = 0.000$, $N_{L.catta} = 13$, $N_{P.verreauxi} = 16$, $P = 0.0001$). However, the number of behavioral transitions per unit of time did not differ between sexes either in *Lemur catta* (Exact Mann-Whitney: $U = 10.500$, $N_{\text{males}} = 7$, $N_{\text{females}} = 6$, $P = 0.154$) or in *Propithecus verrauxi*

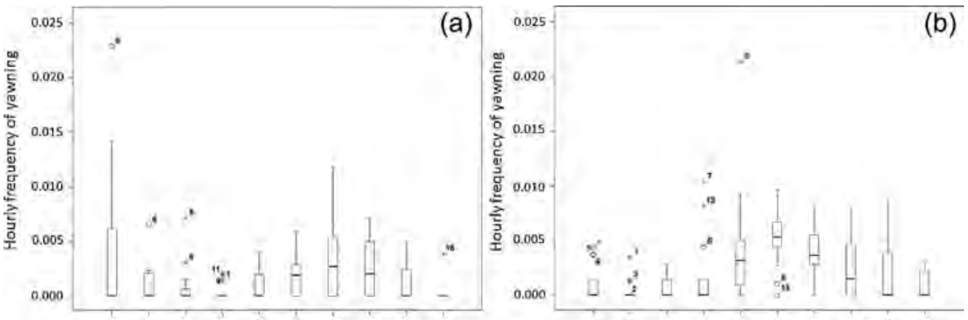

Fig. 2. Daily hourly frequency of the distribution of yawning (from 07.00 am to 05.00 pm) in *Propithecus verreauxi* (a) and *Lemur catta* (b). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range, IQR. The open dot indicates an outlier more than 1.5 IQR from the rest of the scores. Asterisks indicate outliers more than 3 IQR from the rest of the scores.

TABLE I. Lemurs' External Body Temperatures (Three Data Points Per Lemur Collected on Hairless Head Zones via Noncontact Infrared Thermometer Lafayette TRP-19; mean ± SE) in Each Period of the Day (Hourly Intervals)

Species	Time of the day	Temperature (mean ± SE)
Lemur catta	07.00–08.00	24.42 ± 0.92
	08.00–09.00	27.52 ± 1.68
	09.00–10.00	27.14 ± 1.21
	10.00–11.00	28.21 ± 1.41
	11.00–12.00	27.44 ± 0.44
	12.00–13.00	28.32 ± 0.51
	13.00–14.00	26.89 ± 1.19
	14.00–15.00	26.38 ± 0.67
	15.00–16.00	26.11 ± 0.96
	16.00–17.00	26.87 ± 1.59
Propithecus verreauxi	07.00–08.00	22.87 ± 0.66
	08.00–09.00	28.91 ± 1.69
	09.00–10.00	21.78 ± 1.39
	10.00–11.00	28.52 ± 0.25
	11.00–12.00	30.66 ± 0.64
	12.00–13.00	28.29 ± 0.45
	13.00–14.00	27.67 ± 0.34
	14.00–15.00	28.49 ± 0.20
	15.00–16.00	28.22 ± 0.36
	16.00–17.00	–/–

(Exact Mann-Whitney: $U = 23.000$, $N_{\text{males}} = 10$, $N_{\text{females}} = 6$, $P = 0.423$). Yawning was significantly more frequent in *Lemur catta* than in *Propithecus verrauxi* (Exact Mann-Whitney: $U = 0.00$, $N_{L.catta} = 13$, $N_{P.verreauxi} = 16$, $P = 0.0001$; Fig. 4) (Prediction 2c supported).

The Anxiety Hypothesis

Yawning frequency was significantly higher during the first 10 min after exposure to a disturbing event than in the MC condition. No difference was recorded for the second (10-20 min) and third block (20-60) between the two conditions. The same result was obtained for *Propithecus verreauxi* (Exact Wilcoxon Test: $T<_{0\text{-}10\,min} = 105.00$, $ties = 1$, $N = 15$, $P < 0.0001$; $T_{10\text{-}20\,min} = 4.00$, $ties = 11$, $N = 15$, $P = 0.875$; $T_{20\text{-}60min} = 2.00$, $ties = 10$, $N = 15$, $P = 0.188$; Fig. 5a) and *Lemur catta* (Exact Wilcoxon Test: $T<_{0\text{-}10\,min} = 91.00$, $ties = 0$, $N = 13$, $P < 0.001$; $T_{10\text{-}20\,min} = 20.50$, $ties = 6$, $N = 13$, $P = 0.281$; $T_{20\text{-}60min} = 36$, $ties = 4$, $N = 13$, $P = 0.125$; Fig. 5b).

DISCUSSION

The aim of this study was to test various hypotheses of yawning and examine how some morphological, motivational and social factors affect yawning frequencies in lemurs. We first tested whether the lack of sexual dimorphism in *Lemur catta* and *Propithecus verreauxi*, would determine a lack of yawning differences between males and females as predicted by the Dimorphism Hypothesis. Indeed, we found no differences in yawning frequency between males and females in either of these strepshirrine species [Prediction 1 supported].

In many highly sexually dimorphic primates males have longer canines than females, are often dominant and actively defend their groups and territories (e.g., *Macaca fascicularis*, *M. nigra*, *M. fuscata*, *Theropithecus gelada*). In these species, males yawn more frequently than females since they display the so-called "threat yawn" characteristic of aggressive, competitive interactions [Hadidian, 1980; Leone et al., 2014; Troisi et al., 1990]. However, in both *P. verreauxi* and *L. catta* females are dominant [Jolly, 1966; Kappeler, 1997; Norscia & Palagi, 2015]. The canines of the females are used during attacks directed towards both sexes and seasonal peaks of inter-sex aggression have been widely recorded [Pereira & Kappeler, 1997; Vick &

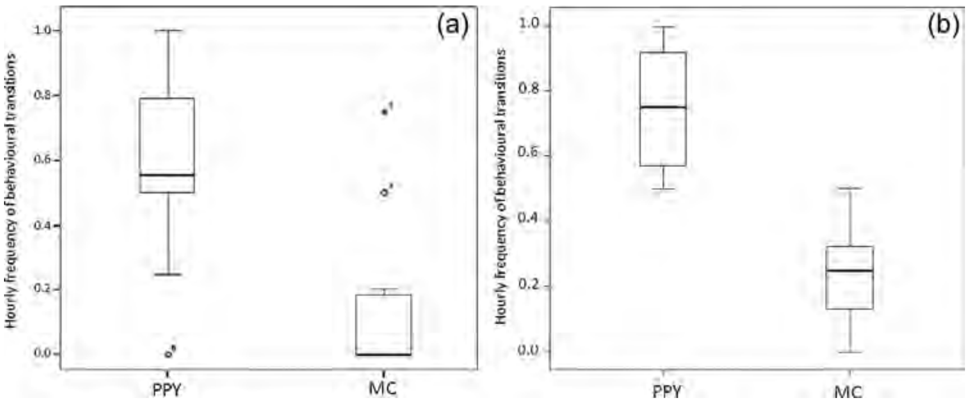

Fig. 3. Frequency of behavioral transitions preceding and following yawns (PPY) and during control condition (MC) recorded in solitary context in *Propithecus verreauxi* (a) and *Lemur catta* (b). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range, IQR. The open dot indicates an outlier more than 1.5 IQR from the rest of the scores. Asterisks indicate outliers more than 3 IQR from the rest of the scores.

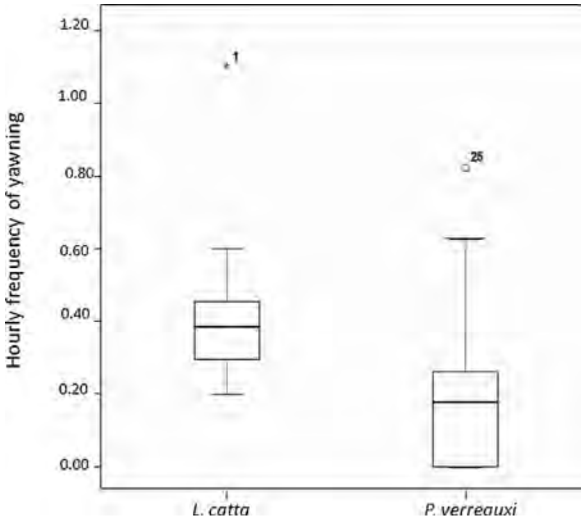

Fig. 4. Hourly yawning frequencies recorded in *L. catta* and *P. verreauxi.* The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range, IQR. The open dot indicates an outlier more than 1.5 IQR from the rest of the scores. Asterisks indicate outliers more than 3 IQR from the rest of the scores.

Pereira, 1989]. In an evolutionary perspective, the high level of both intra- and inter-sexual competition may have resulted in reduced morphological differences between sexes [Kappeler, 1997; Plavcan & van Schaik, 1999]. As expected, in *Propithecus verreauxi* and *Lemur catta* the hourly frequency of yawns did not differ between males and females. Our results are in agreement with previous findings in species with low sexual dimorphism, i.e. *Homo sapiens* [Schino & Aureli, 1989]*, Pan troglodytes* [Vick & Paukner, 2010] and *Pan paniscus* [Demuru & Palagi, 2012], in which no sex difference in yawning frequency were reported.

According to the *State Changing Hypothesis* [Provine, 2005] yawning, like other self-directed behaviors such as scratching and body shaking, is associated with neural mechanisms related to arousal. Yawns may stir up an individual's physiology thus being associated to the transition from one behavior to another. From this perspective, yawning can be considered as a displacement behavior [Tinbergen, 1952]. The association between yawning and behavioral transitions, including sleep/wake cycle, has been demonstrated in several primate species including humans, macaques, hamadryads [Hadidian, 1980; Kummer, 1968; Maestipieri et al., 1992; Troisi et al., 1990] and, recently, in geladas [Leone et al., 2014]. In *Lemur catta* and *Propithecus verreauxi* the frequency of yawns around behavioral transitions was significantly higher than when there was no transition, independent of any audience effect (defined as the presence of another subject within two meters from the yawner) (Prediction 2a supported). In humans, yawning is associated with increased activity levels even outside the context of waking/sleeping [Beanninger et al., 1996]. In chimpanzees yawning is related to a change in general activity levels [Vick & Paukner, 2010]. Displacement behaviors, and specifically self-scratching, were shown to increase sharply around behavioral state changes in *Lemur catta* [Buckley & Semple, 2012]. Our data on yawning show the same trend: yawning punctuates changes in general activity levels of individuals.

The frequency of yawns in the two lemur species differed strongly and was strictly related to their activity level. *Lemur catta*, characterized by higher levels of basal activity (defined as behavioral transitions per unit of time) also yawned significantly more frequently than *Propithecus verreauxi* (Prediction 2b supported). Even though the two study species show some similarities—i.e., phylogenetic closeness, sharing of the same environment (to the extent that animals living in the same habitat in the Berenty Reserve often feed on the same tree)—their ecology differs. The frugivorous/

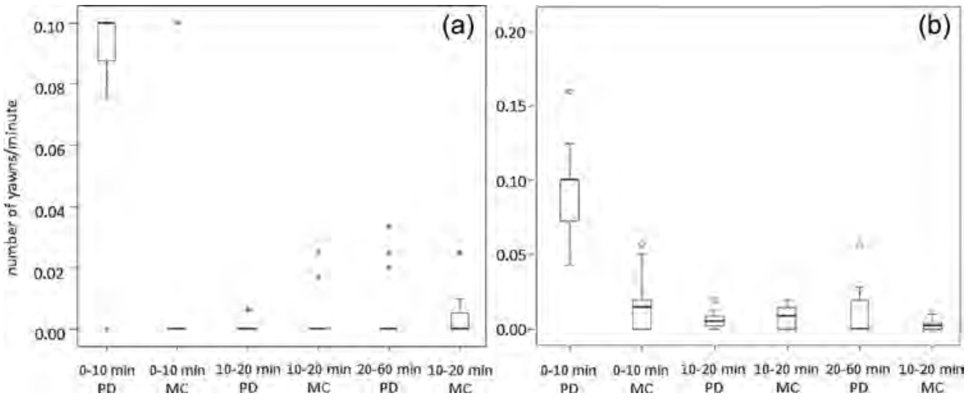

Fig. 5. Yawning frequency in *Propithecus verreauxi* (a) and *Lemur catta* (b) in the three time blocks (0-10min; 10-20min; 20-60min) in Post Distress condition (PD) and in Matched-Control condition (MC). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range, IQR. The open dot indicates an outlier more than 1.5 IQR from the rest of the scores.

omnivorous *L. catta* (in Berenty, the individuals of these species can hunt grasshoppers, spiders and cicadas) have a more dynamic life-style characterized by an active search and competition for food [Jolly, 1966] whereas the folivorous *P. verreauxi* spend more time feeding and a large part of the day resting for digestion [Jolly, 1966; Norscia et al., 2006]. Our analysis on the behavioral transitions per unit of time clearly demonstrates more frequent behavioral shifts and more frequent yawns in *L. catta* than *P. verreauxi* as predicted by the *State Change Hypothesis* [Baenninger, 1997] (Prediction 2c supported).

The *Anxiety Hypothesis* predicts that environmental and social stressors can induce yawning. Chimpanzees and gorillas were reported to yawn in the proximity of human observers [van Lawick-Goodall, 1968; Nishida, 1970; Schaller, 1963]. In *Macaca nigra*, yawns were reported to occur in contexts that elicited anxiety [Hadidian, 1980], and in captive *Macaca silenus* yawning increased in the presence of visitors [Mallapur et al., 2005]. *Lemur catta* was observed yawning during agonistic scent-marking displays [Jolly, 1966] even though the author was not able to clearly associate yawning with a specific context. Roeder et al. [1994] described yawning in *L. catta* as temporally associated with stressful encounters. However, these reports are mostly anecdotal. Indeed, our findings provide the first empirical evidence of a direct connection between potential stressors and the yawning response in lemurs. Both *Lemur catta* and *Propithecus verreauxi* yawned within 10 minutes of exposure to a disturbing event (Prediction 3 supported). This finding contrasts with literature on non-primates showing a 20–40 min delayed yawning response to stressful stimuli, such as isolation [*Sula granti*, Liang et al., 2015], confinement and handling [*Melopsittacus undulatus;* Miller et al., 2010] and electric shocks [*Rattus norvegicus*; Moyaho & Valencia, 2002]. In these studies, the delayed response is explained through the *Arousal Reduction Hypothesis*, predicting that yawning is elicited by arousal reduction, when the animal starts relaxing. Our results do not challenge the *Arousal Reduction Hypothesis* because the study setting and the nature of stressing stimuli were different from those of previous studies. Lemurs were observed in their natural environment and everyday social stimuli. Therefore, we hypothesize that the arousal provoked by natural, familiar stimuli is usually milder than that caused by extraneous, infrequently encountered stimuli. Additionally, in the wild, animals can minimize their exposure to stressors by escaping. This can lower the arousal response, meaning that animals in their natural habitat can recover from some arousal increases (stress) faster than their laboratory counterparts.

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