HEALTH STATUS AND NESTING PATTERN IN MALE GRAY MOUSE LEMURS
(MICROCEBUS MURINUS)

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Résumé.— Condition corporelle et grégarisme diurne chez des mâles Microcèbes (Microcebus murinus) en saison de reproduction.— Chez un primate prosimien malgache nocturne, le regroupement dans des gîtes pendant la période de sommeil diurne représente un avantage énergétique certain. Sur des mâles microcèbes captifs maintenus en groupes sociaux, nous avons testé l’hypothèse que les mâles présenteraient des paramètres physiologiques différents en fonction de leur patron de regroupement dans les gîtes au début de la saison de reproduction. La composition des gîtes, l’état de santé à travers le poids corporel, la fonction de reproduction (taille des testicules) et le niveau du système immunitaire (nombre de lymphocytes) ont été suivis au début de la période de reproduction et après 4 semaines sur 4 groupes sociaux nouvellement formés de 5 mâles chacun. Un seul gîte était occupé par 3 mâles ou plus dans 85 % des cas, et 14 cas de mâles isolés dans un gîte furent enregistrés, répartis sur 65 % des observations. Les mâles trouvés isolés ou par paires ont perdu significativement plus de poids sur les 4 semaines de suivi que les mâles regroupés par 3 ou plus dans un gîte. En outre, les mâles rejetés des nichoirs communs présentaient un système immunitaire moins bon avant la mise en groupe sociaux par rapport aux mâles regroupés dans les nids. Cependant, aucune évolution significative n’a été observée au cours des 4 semaines chez les microcèbes regroupés dans les gîtes, alors que les mâles isolés ont montré une augmentation significative de leur nombre de lymphocytes. Ces résultats indiquent que, chez le Microcèbe, le regroupement dans les gîtes diurnes pendant la période d’activation sexuelle est un processus complexe, impliquant différentes conséquences énergétiques et immunitaires.

Summary.— In the Gray Mouse Lemur, a nocturnal Malagasy primate, nest sharing during the diurnal sleeping period provides significant thermoregulatory advantages. Using socially housed captive male mouse lemurs, we tested the hypothesis that males would present differential physiological parameters depending on their nest sharing patterns at the beginning of the breeding season. Nest composition, and health status inferred from body weight, reproductive activity (testis width) and level of immune activity (lymphocyte number) were recorded at the beginning of the breeding season and after 4 weeks in newly formed 4 groups of 5 adult males. One nest was occupied by 3 males or more in 85 % of the surveys, and 14 cases of males found alone in a nest were recorded, distributed over 65 % of the surveys. Males found alone or in pairs lost significantly more weight after the 4 weeks of social grouping than males found in groups of 3 or more individuals in a nest. Additionally, males expelled from common nests had reduced immune activity prior to social grouping than nest associated males. Nest associated males had little change in their lymphocyte numbers after the 4 weeks of social grouping, whereas isolated males exhibited a pronounced increase in lymphocyte numbers. These results indicate that, in mouse lemur, nest-sharing pattern at the beginning of the breeding season is a complex process, yielding diverse energetic and immunological consequences.

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— 197 —
Within mammals, including primates, sociality plays a crucial role in optimizing individual fitness, by reducing predation risks and minimizing social conflicts (Ebensperger, 2001). In primates, social relationships are a major determinant of male individual fitness, but the benefits of sociality in the absence of a cohesive social structure are less obvious. This is the case for many nocturnal primates, which are usually considered to be primarily solitary animals (Charles-Dominique, 1995). Among social behaviours, gathering in nests is known to provide energetic advantages. It reduces the amount of heat lost from the body by decreasing the exposed surface, and generates local heating (Andrews et al., 1987; Contreras, 1984; Hayes et al., 1992; Springer et al., 1981). Moreover, gathering in sleeping sites is a common feature in primates, which spend about half of their lives at sleeping sites and use this behaviour to provide safety from predators as well as physical comfort (Isbell, 1994; Bearder et al., 2003).

Most nocturnal prosimian primates are believed to be solitary and to exhibit the most primitive form of sociality (for review, see Muller & Thalmann, 2000), but their social systems seem to be more complex (Schuelke & Kappeler, 2003; Gursky, 2005; Nekaris, 2006). Endemic to Madagascar, Gray Mouse Lemurs (Microcebus murinus) deviate from predictions of sexual selection theory among solitary lemurs by varying their reproductive tactics or social organization in response to variation in the availability and distribution of local resources (Kappeler, 1993; Fietz, 1998; Schmid & Kappeler, 1998; Radespiel, 2000; Eberle & Kappeler, 2002). To cope with seasonal variations in climate and food resources, mouse lemurs have developed various adaptations that have been studied both in the wild and in captivity. First, mouse lemurs exhibit highly seasonal variations in their metabolic and reproductive functions, with fattening and sexual rest during autumn and winter, and a breeding season restricted to spring and summer (Schmid & Kappeler, 1998; Perret & Aujard, 2001). These physiological variations are strictly dependent on the annual variation in photoperiod and can thus be reproduced in captive conditions under artificial lighting (Perret et al., 1998; Perret & Aujard, 2001). Secondly, one particular characteristic of this species is the practice of daily torpor, a rare trait within primates (Ortmann et al., 1997; Schmid, 2000; Perret & Aujard, 2001). The pattern of daily torpor also shows seasonal variations and contributes greatly to energy-saving in mouse lemurs (Aujard et al., 1998; Séguy & Perret, 2005; Terrien et al., 2008). Finally, even though mouse lemurs are generally seen alone during their nocturnal activities and are therefore associated with a “solitary foraging” pattern, they also gather in tree holes during their diurnal sleep period (Schmid, 1998; Radespiel et al., 1998).

Field studies on sleeping groups of mouse lemurs have shown that the composition of these sleeping associations depends on the sex of their members (Radespiel et al., 1998, 2003), with most associations composed of closely related females, while males were usually found alone or in pairs during the mating season (Dammhahn & Kappeler, 2008; Lahann, 2008). The influence of environmental factors such as the season suggests that sleeping associations are a potentially adaptive response to environmental constraints. In addition, research in captivity has shown that this gregarious behaviour provides energetic benefits to the animals by lowering their resting metabolic rate and increasing the ambient temperature in the nest (Perret, 1998; Séguy & Perret, 2005). Under the controlled conditions of captivity, the optimal amount of energy is saved when three animals nest together (Perret, 1998). Moreover, when presented with a choice between nests at different ambient temperatures, mouse lemurs specifically choose a comfortable temperature for their diurnal sleeping period (Aujard et al., 2006).

We studied the nest sharing pattern of captive male mouse lemurs maintained in social groups over the first 4 weeks following the shift from a short photoperiod to a long photoperiod, corresponding to the beginning of the breeding season in nature. In captivity, this period is characterized by behavioural and physiological changes. Basically, males exhibit an increase in testosterone levels and a decrease in body weight (Perret, 1992, 1995; Perret & Aujard, 2001). Under captive conditions, animals are fed ad libitum and are maintained at a constant ambient temperature, leading to relatively good health conditions compared to animals in the wild. But group-housed males cannot avoid social contacts during the night and can only avoid each other by sleeping in different nests. Consequently, the objective of this study was to determine the physiological parameters associated with nesting pattern in socially-housed male Gray Mouse Lemurs.
MATERIALS AND METHODS

SUBJECTS AND HOUSING

All mouse lemurs used in this study were born in the laboratory breeding colony (Brunoy, MNHN, France, European Institutions Agreement No. A91-114-1). General conditions of captivity were maintained constant with respect to ambient temperature (25°C), humidity (55 %), and food (fresh fruits, milk porridge and insects) available ad libitum. In captivity, seasonal variations in physiological functions can be entrained by alternating 6-month periods of long photoperiod (14 h of light / day, LP) and short photoperiod (10 h of light / day, SP) under artificial light (fluorescent tubes during the day and dim red light during the night). Twenty adult male mouse lemurs were studied (mean age ± SEM: 2.8 ± 0.5 years), that were not related to each other for at least three generations.

All experiments were carried out in accordance with the European Communities Council Directive (86/609/ EEC). All efforts were made to minimize animal suffering throughout the experiments.

EXPERIMENTAL PROCEDURE

We focused our experiments on the beginning of the breeding season known to occur after the onset of exposure to long photoperiod in captivity (Perret, 1992). In order to avoid the influence of any social stress prior to the experiment, we established social groups with animals that had never been in contact before and that had been in smaller groups (no more than 3 animals per group) for the duration of the previous short photoperiod. Four groups were constituted, each consisting of 5 males. All animals were put together on the day prior to the photoperiodic shift (week 0) and were studied over the following 4 weeks. Each group was housed in a cage (8 m²) provided with branches and nests. Nests were made of wood and their volume (1000 cm³) provided enough room for all animals of each group to sleep together if they so wished. All nests were placed in similar positions with regard to their height above ground and their orientation with respect to the light source. The number of nests was equal to the number of animals.

EVALUATION OF NEST COMPOSITION AND BIOLOGICAL MEASUREMENTS

We measured the body weight and testis width of each male before and after the 4 weeks of social grouping. In addition, we noted daily group composition in each nest, from week 0 to week 4. Records were obtained during the second half of the diurnal phase when animals were in their (diurnal) resting phase. Body weight was estimated using a balance (precision ± 1 g). Testis width was measured through the scrotum with hand-calipers (precision ± 1 mm) and was expressed as the sum of both testes sizes. We calculated an association index (AI) for each male throughout the experiment, based on the number of males found in the nest of each animal divided by the total number of males that could be found in this nest if all males nested together. The AI is close to 1 if the animal has been found sleeping with the majority of the other males throughout the experiment, whereas an AI close to 0 indicates an animal found sleeping alone most of the time. Males are considered to have an associated pattern if their AI is above the average value, and males with AI below the average value are considered to have an isolated pattern. In addition, blood from the saphenous vein was drawn from each male without anesthesia by venipuncture, before the experiment (week 0) and at the end of the experiment (week 4). Lymphocyte counts were made from thin smears of a 10 μl drop of blood, because these cell populations are implicated in the immune defense of the organism and are thus indicators of health condition (Huppert et al., 1998; Davis et al., 2008). Within a normal range, a decrease in lymphocyte numbers is associated with a reduction in immune activity and thus in the potential defense of the organism against pathogens. Blood smears were air dried, fixed with methanol and stained with Giemsa stain (Sigma, Saint Quentin, France). Counting of lymphocytes was carried out in 6 random optical fields / slide by making blind assessments. Blood cell data are presented as absolute numbers (per optical field = OF) since smears were of relatively even thickness.

STATISTICAL ANALYSES

Single comparisons between two groups were performed using Student’s t tests (Systat 11 for Windows) on related or unrelated measures and the evolution of body mass and of testes size was assessed using ANOVA for repeated measures. The distribution of nest association over the 4-week period of experimentation was analyzed using a χ² test. Correlation coefficients (Pearson’s) were used to ascertain whether biological parameters measured before or after social housing varied with the association index. All data are expressed as mean ± SEM. Differences between groups were considered to be statistically significant when P < 0.05.

RESULTS

Animals never gathered all together, except in one case recorded in the first week following social grouping. One nest was occupied by 3 males or more in 85 % of the surveys. Throughout the experiment, 14 cases of isolated males were recorded (mainly observed in 6 individuals out of 20), distributed on 65 % of the surveys. In fewer cases, males were found in pairs. The nest distribution did not differ between the 4 consecutive weeks (χ² = 1.67, d.f. = 3,
Because no significant change was observed between the first two and the second two weeks ($F_{1/19} = 0.02$, NS), we calculated the association index (AI) of each male over the total experimentation period. The average AI was $0.41 \pm 0.02$ (range $0.25 - 0.56$, $N = 20$). Because males almost never nested together, the maximum AI is far from 1.

In all groups studied, typical physiological changes were observed in the 4 weeks following exposure to long days. Body weight significantly decreased between week 0 and week 4 ($F_{4/76} = 12.1$, $P < 0.001$). Body weight averaged $105 \pm 3$ g before the onset of long days and $87 \pm 3$ g at the end of the experiment ($F_{1/19} = 15.6$, $P < 0.001$). When considering the relation between AI and body weight before group housing, no significant relationship was found between these two parameters ($r = -0.112$, d.f. = 18, $P > 0.05$ – Fig. 1). However, when considering the AI estimated over the 4-week period and physiological measures at week 4, we found a significant relationship between AI and body weight at week 4 ($r = 0.540$, d.f. = 18, $P < 0.02$). We then considered the nesting pattern of each male (associated or isolated) to determine whether differential changes in physiological parameters occurred. The present results revealed that isolated males lost significantly more weight over the 1-month period than nest-associated males did ($t = -2.2$, d.f. = 18, $P < 0.05$). Associated males lost on average 9 % of their initial body weight, while isolated males lost more than 23 %, for similar body weights at the time of group housing 4 weeks before (Tab. I).

![Figure 1.— Individual association index (AI) of male mouse lemurs (N = 20) during the 4 weeks of social housing in relation to body weight (g) prior to group formation (week 0) and after 4 weeks of social housing (week 4). The AI is close to 0 when the frequency of rejection from common nests is high.**: $P < 0.02$.](image-url)
TABLE I

Values of body weight (g) and lymphocyte numbers (cells/optical field) of socially housed male mouse lemurs. Values are given according to the nesting pattern of the animals. Associated males were mostly found sleeping with at least 2 other males (high association index), isolated males were mostly found sleeping alone or in pairs (low association index). Data indicate mean values (± SEM) prior to and after 4 weeks of social grouping. The statistical analysis compared the evolution of each parameter between the 2 categories of animals.

<table>
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<tr>
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<th>Associated males (N = 10)</th>
<th>Isolated males (N = 10)</th>
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<td></td>
<td>Week 0</td>
<td>Week 4</td>
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<tr>
<td>Body weight</td>
<td>102 ± 3</td>
<td>93 ± 4</td>
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<tr>
<td>Lymphocyte number</td>
<td>31.4 ± 2.8</td>
<td>24.9 ± 6.4</td>
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Figure 2.— Correlation between the individual association index (AI) of male mouse lemurs (N = 20) during the 4 weeks of social grouping and lymphocyte counts (cells/optical field) prior to group formation (week 0) and after 4 weeks of social housing (week 4). The AI is close to 0 when the frequency of rejection from common nests is high. *: P < 0.05.
Testis width was on average 16.1 ± 0.9 mm at week 0 and gradually increased after the onset of long days to reach an average value of 20.8 ± 0.4 mm ($F_{4/76} = 54.9, P < 0.001$). No significant relationship was found between AI and testis size at week 4 ($r = 0.295, d.f. = 18, P > 0.05$) or between body weight and testis size at week 4 ($r = -0.097, d.f. = 18, P > 0.05$).

Lymphocyte counts at week 0 were significantly correlated with AI ($r = 0.505, d.f. = 18, P < 0.05 – Fig. 2$). A lower amount of circulating lymphocytes was observed before group housing in animals that were to be found alone in nests. No significant relationship could be found between body weight and lymphocyte numbers at week 0 ($r = -0.067, d.f. = 18, P > 0.05$). Lymphocyte numbers did not vary significantly from week 0 to week 4 ($t = -0.4, d.f. = 19, P > 0.05$). Lymphocyte numbers at week 4 were not correlated with body weight ($r = -0.053, d.f. = 18, P > 0.05$) or testis width ($r = -0.208, d.f. = 18, P > 0.05$). Moreover, lymphocyte counts at the end of the experiments were not significantly correlated with AI ($r = -0.212, d.f. = 18, P > 0.05$). This lack of correlation could be explained by a differential development of lymphocytes according to the nest association pattern of each male (Tab. 1). Indeed, the change in lymphocyte counts in isolated males was significantly different from the change in lymphocyte counts observed in associated males ($t = -3.0, d.f. = 18, P < 0.01$). For this cell line, no significant difference was found between week 0 and week 4 in associated males ($t = 1.1, d.f. = 9, P > 0.05$), whereas a significant increase ($t = -2.3, d.f. = 9, P < 0.05$) was found between week 0 and week 4 in isolated males.

**DISCUSSION**

Socially-housed male mouse lemurs presented different physiological characteristics depending on the number of males with which they were found associated in sleeping nests. Under captive conditions, at the beginning of the breeding season induced by exposure to long photoperiod, males exhibit an increase in testosterone levels and a decrease in body weight (Perret, 1992, 1995; Perret & Aujard, 2001).

Our data showed that when five males were housed together, three or four males out of five gathered in the same nest, while other males were found alone or in pairs during the diurnal sleeping period. A previous study (Perret, 1998) showed that maximal energetic benefit was achieved when three animals nested together. It is not surprising then that a three-male association was most commonly observed in our study. Males found sleeping alone or in pairs lost more weight than males sleeping with three or more conspecifics after four weeks of social grouping, and different variations in lymphocyte numbers were observed. In nature, the weight loss is currently associated to intense mating activity but in our experiment, males were not in sexual competition since no females were available. Likewise, loss of body mass cannot be attributed to a limited access to food because food was provided in quantity above requirements. Two potential explanations can be considered: a lack of energy saving or a syndrome due to social stress. The importance of reaching a temperature of comfort within the nest has been demonstrated recently (Aujard et al., 2006) leading to energy saving through thermoregulatory processes. However, it is unlikely that males nesting alone or by pairs have high energetic disadvantage owing to the fact that in captivity, ambient temperature is maintained constant. In the wild, where animals are exposed to drastic seasonal variation in food availability especially at the beginning of the breeding season, the effect of social exclusion on energetic loss may be more dramatic and may influence health condition and survival.

A stress syndrome leading to body weight loss in males nesting alone cannot be excluded and it would be interesting to measure cortisol levels in these animals. In primates, cortisol levels were generally associated to the type of social grouping, the frequency of agonistic interactions or competition for resources (Albert et al., 1992; Stoinski et al., 2002; Pride, 1995). In mouse lemurs, cortisol levels in plasma measured during the breeding season in males maintained with females have been demonstrated to be two-fold higher than those recorded in males maintained isolated. This suggests that living in group leads to increased social stress whatever the social position of males (Perret & Predines, 1984). In our study, we found a significant
increase in the lymphocyte numbers of males sleeping alone compared to associated males after four weeks of group housing. It cannot be excluded that sleeping alone may present advantages in some particular conditions and on a long-term basis. Recently, data on rodents have demonstrated that social interactions can lead to an increase in corticosterone levels and in the propensity to develop tumors (Azpiroz et al., 2008). In humans also, immunity seems to be temporarily compromised to maintain social relationships (Segerstrom, 2008). If considering that nest sharing in mouse lemurs is part of a more general social network, it can be hypothesized that maintenance of this social network is costly in energy, and could impair immunity. Nest sharing would represent a compromise between energy saving strategies and preservation of a good immune function.

Like in higher primates, where gregariousness and group size are influenced by females’ reproductive cycle (Nunn, 1999; Matsumoto-Oda, 1999), the social organization of strepsirhines can be influenced by male hormonal levels or female reproductive status (Kappeler, 1993). Indeed, a lower survival rate of males was demonstrated during the mating season in wild mouse lemurs (Kraus et al., 2008). In captive mouse lemurs, the short day period, corresponding in the wild to the winter dry season, is characterized by a complete sexual rest, where plasma testosterone levels drop to a basal level in all males. In captivity, sexual arousal, male-male competition and agonistic behaviours increase drastically after exposure to a long photoperiod, but only if males are in the presence of sexually active females (Perret, 1992). In this condition, competition between males occurs in the first weeks of long days and results in a reduction of testosterone levels (and testis width) in subordinate males during the mating period via a pheromonal effect (Perret, 1995). In the present study, males were isolated from females and as expected we found no difference in the testis widths of the studied animals and no correlation between testis width and association in the nests.

In captivity, all mouse lemurs gain weight during the short day period. No significant difference in body weight was found between animals prior to the onset of exposure to a long photoperiod. But at this time, differences in lymphocyte counts could be already observed between the animals. Our data showed that males that were found sleeping alone in nests during the period of sexual activation were those with reduced immune system prior to social grouping. The reduction in immune competence associated with social stress is well documented in rodents (Dréau et al., 1999; Stephanski & Engler, 1999) and primates (Alberts et al., 1992; Gust et al., 1993), but the attempt to establish a relation between immune measures and the social position of individuals in newly formed groups of macaques was unsuccessful (Clarke et al., 1996). In mouse lemurs, males expelled from common nests were characterized by lower levels of lymphocytes before the experiment, suggesting that mouse lemurs may have the ability to detect the health status of their conspecifics. A weak immune system can be the origin or the consequence of a poor resistance to pathogens, reducing individual fitness and survival. It is therefore important for an individual to be aware of the health performance of its conspecifics, especially in the context of sexual selection (Johnstone, 1995; Nunn et al., 2000; Schwensow et al., 2008). In many polygamous species, females can identify the dominance status of males from chemical cues (Johnston & Rasmussen, 1984; Drickamer, 1992; Horne & Ylönén, 1996) and are able to discriminate male body condition from odours (Kavalier & Colwell, 1995; Ferkin et al., 1997). From what is known about pheromones in the mouse lemur (Schilling & Perret, 1987), it is likely that information on health status will be conveyed by urinary components in this species.

In conclusion, this study demonstrates that nest-sharing pattern in the male Gray Mouse Lemur is associated with changes at the physiological level. We believe that the importance of such a social behaviour as an adaptive response to environmental constraints has been underestimated, and that the use of detailed behavioural observations will help clarify the remaining open questions.

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