Benefits and costs of group-sleeping in *Microcebus murinus*: sleeping site ecology and parasitism

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ABSTRACT

The transition from a solitary to a group life-style is one of the major transitions in evolution. Group-living individuals might benefit from a better access to resources, such as food or sleeping site, a reduced predation risk and cooperative breeding. However, they also face costs associated to sociality such as a higher risk of parasite transmission. In this study we compared the sleeping site ecology and the level of parasitism between group-sleeping and solitary females in gray mouse lemurs (*Microcebus murinus*) to investigate whether group-sleeping females benefit from better quality sleeping site and harbor more parasites than solitary females. We equipped 31 females with a radiocollar during two field seasons in Kirindy forest (Madagascar) allowing the localization of their sleeping sites, the recording of the sleeping sites' characteristics and the determination of the sleeping condition, i.e. solitary or group-sleeping, of the females. Moreover, we counted ectoparasites and collected and analysed fecal samples from these females. Several differences in the characteristics and usage pattern of the sleeping sites between group-sleeping and solitary females were found. Group-sleeping females slept on average in tree holes located higher above the ground and in larger and more often living trees, which should provide a better thermoregulation and protection against predators. These results suggest that there may be competition over sleeping and that group-sleeping females used higher quality sleeping sites compared to solitary females. However, while group-sleeping females did not harbor more parasites than solitary ones, we found that group size was positively correlated with parasitism within groups: larger groups harbored more ticks and more gastro-intestinal parasite species than small groups. Our results suggest that group-sleeping provided a benefit in term of higher quality sleeping sites but that large group may suffer from a parasites cost associated to sociality.

CONTENT

INTRODUCTION

Benefits and costs of sociality

The transition from solitary to group-living is considered to be one of the major transitions in evolution (Maynard-Smith and Szathmary 1995). Group-living social organization has evolved independently multiple times from a solitary ancestor in several distinct taxonomic groups (Arnold and Owens, 1998; Nowak et al., 2010; Shultz et al., 2011). This type of social organization can be defined by a stable association of more than two adult individuals that interact more often with each other than with other individuals outside the group (Kappeler and van Schaik, 2002). These spatio-temporal associations with conspecifics are thought to have evolved due to fitness benefits.

Better access to resources and reduced predation risk are seen as the major ecological benefits gained by individuals forming group association (Clutton-Brock and Janson, 2012). Resources are defined as substances or objects in the environment that can be used by individuals to promote their growth, survival and reproduction (Radespiel et al., 1998). While food resources have been historically regarded as the main resource in the light of benefits of sociality, shelters or sleeping sites also represent an important resource that have been only lately considered (Lutermann et al., 2006). The choice of the sleeping sites can have, indeed, profound consequences on individuals' fitness (Karanewsky and Wright, 2015): in different species of mammals and birds, the availability and the quality of tree holes are correlated with survival or reproductive success of individuals (Isaac et al., 2008). Individuals living in groups also benefit from a reduced predation risk (Clutton-Brock and Janson, 2012; Schneider and Kappeler, 2016), especially diurnal species that face to a higher predation risk (Shultz et al., 2011; Santini et al., 2015). Both in the context of communal defence of resources and defence from predation, individuals can further benefit, in term of inclusive fitness, if the group is kin based as the individuals' interests will match the one of the other individuals of group. Moreover, individuals that form group could increase their inclusive fitness by helping their close relatives in parental care (e.g. cooperative breeding). Kin selection, indeed, has been recognized to be a major mechanism favouring the evolution of sociality (Hamilton, 1964; West Eberhard, 1975).

Group-living individuals also face some costs associated to sociality (Hamilton 1987), such as intragroup competition over resources, especially in large groups, with possible negative consequences on stress, body condition, breeding success, and survival (Clutton-Brock and Janson, 2012). Moreover, social species are exposed to a higher parasite transmission risk (Alexander, 1974). Parasites are defined as organisms that live at the expense of a larger individual of another species, called the host (Anderson and May, 1979). By consuming the host resources, parasites can lead to different costs such as a loss of the energy allocated to the immune system, involving a higher susceptibility to other parasite infection and stress, or a reduction in

competitive abilities impacting ultimately the host's survival and reproductive outcome (O'Donnel, 1997). Group living generates a fertile ground for parasite transmission: high density of hosts and frequent social and sexual interactions between conspecifics increase the exposure to parasites and provide opportunities for their spread (Snaith et al., 2008). Moreover, genetically-related individuals that often live in a same group are likely to share similar susceptibility characteristics which further facilitates parasite spread (Shykoff and Schmid-Hempel, 1991). Furthermore, it has been suggested that the intensity of parasitism within a group is related to its size (Patterson and Ruckstuhl, 2013). Accordingly, individuals living in bigger groups harbor a higher parasite intensity or diversity compared to individuals living in smaller groups (Cote and Poulin, 1995). Similar results were revealed in meta-analyses in birds, mammals and primates (Vitone et al., 2004; Rifkin et al., 2012; Patterson and Ruckstuhl, 2013). Consequently, parasitism represents one of the major cost of group-living.

Some particular adaptations in social species may further influence the balance between benefits and costs associated to sociality. In particular, group-living species have evolved anti-parasite defense to avoid parasite or mitigate the cost of parasite infestation. Accordingly, it has been shown that social species invest more in the immune system than solitary species (Møller et al., 2001). In addition to the immune system, pathogen-mediated selection has driven the evolution of a sophisticated set of behavioral parasite avoidance strategies (Schaller and Duncan, 2007). This "behavioral immune system" can act as a first line of defense, before the activation of the immune system, to reduce or avoid contact with parasites or, once the parasite infection occurred, to accelerate the removal and recovery (Schaller and Duncan, 2007; Schaller and Park, 2011). One well known example is represented by grooming, a widespread social behavior in mammals and birds, which has been shown to reduce the intensity of ectoparasite infections in several species (Mooring et al., 1996, 2004). This set of behaviors could explain the absence of a positive relationship or even the negative relationship between parasite infection and group size that has been found in some social species (Lett Frédéric Bordes et al., 2007; Snaith et al., 2008). A better evaluation of the trade-offs between benefits and costs of group living could improve our understanding on the determinants driving group association and ultimately the evolution of sociality.

Gray mouse lemurs (Microcebus murinus) represent an exceptional model system to study benefits and costs of sociality in primates. Indeed, mouse lemur is considered to be a primate with primitive social tendencies and displays a rare form of facultative sociality (Lutermann et al., 2006). While males and some females sleep alone during the day, other females form day time sleeping associations (Wimmer et al., 2002; Eberle and Kappeler, 2006). Moreover, this species show characteristics that are related to a solitary lifestyle and are thought to be a retention from the common primate ancestor: they forage solitarily, are nocturnal and small (Lutermann et al., 2006). Therefore, the life-style of gray mouse lemurs appears to fall between ancestral solitary life style and sociality. These key-features provide a unique occasion to evaluate

the benefits and costs involved in the evolution of sociality in gray mouse lemurs, and ultimately in primates in general. In this study in mouse lemurs, I compared solitary females and "group-sleeping females" (i.e., females that form sleeping associations during the day) to investigate (1) whether group-sleeping females had access to better sleeping sites, compared to solitary females and (2) whether group-sleeping females harbored more parasites than solitary females, and/or exhibited some parasite avoidance strategies to decrease the exposure to parasites.

Sleeping condition and sleeping sites

Gray mouse lemurs are arboreal, nocturnal strepsirrhine primates endemic to Madagascar living in the dry deciduous forests of the southern and western areas of the island (Mittermeier et al., 1994). The sleeping groups have a stable composition over time even when changing from one sleeping site to another one (Radespiel, 2000). Occasionally, females of the group can sleep alone in a different sleeping site before returning one or few days later with the group (Mosna and Socias Martinez unpublished data). The size of the sleeping group is usually between two and four females during the dry season (Radespiel, 2000; Eberle and Kappeler, 2006). Sleeping groups have been shown to consist of genetically related females, often one or several closely related dyads (Radespiel et al., 2001). On the contrary, solitary females do not have close females relatives in the proximity (Radespiel et al., 2001). Except for anecdotal reports of gray mouse lemurs sleeping in leaf-nests (Schmid, 1998), female in groups as well as solitary females use tree holes as sleeping sites.

Several factors have been proposed to influence sleeping site selection, as sleeping sites may provide a buffer against fluctuation of external temperatures and a protection against predators (Radespiel et al., 2001). Mouse lemurs are subjected to these ecological pressures: they are exposed to fluctuating temperatures and experience a high risk of predation. Both, the sleeping site characteristics and the patterns of use have been considered in the light of predator avoidance strategies. In particular, characteristics of tree holes such as height or tree diameter have been related to protection against terrestrial predators in many primates (Cheyne et al., 2013; Stewart and Pruetz, 2013), whereas changing often the sleeping site can decrease the likelihood of being tracked by predators. Moreover, in an environment with a high daily fluctuation of temperature, an insulating shelter can provide a buffer against high or low temperatures allowing energy savings (Radespiel et al., 2003). *Microcebus murinus* belong to one of the only two primate genera known to enter in torpor in response to harsh conditions (Schmid, 1998). Gray mouse lemurs, trough daily torpor, decrease their daily energy cost by about 38% and they can maintain torpor until the ambient temperature reaches the threshold of 28°C (Schmid, 1998). Tree holes can provide, through insulation, a microclimate that will delay the time at which the temperature inside reaches this threshold, allowing mouse lemurs to maintain torpor for longer time and thus, reducing

metabolic costs. The insulation capacity has been measured as a difference between the internal and external temperature of the sleeping site. Higher differences represent higher degrees of insulation. Different characteristics of the sleeping site have been studied to understand which factors are related to insulation. A first study in the deciduous forest of Kirindy (Madagascar) found that shelters in living trees present a higher mean insulation capacity compared to shelters in dead trees, indicating a better insulation capacity offered by living trees (Schmid, 1998). Moreover, there is evidence showing that sleeping sites located higher in the tree or in larger trees (larger diameter at breast height, DBH) provide a cooler and less variable microclimate (Isaac et al., 2008). Sleeping sites have been suggested to be a limiting resource for gray mouse lemurs (Radespiel et al., 1998; Schmid, 1998; Dammhahn and Kappeler, 2009; Lutermann et al., 2010). First, the percentage of preferred trees used by females was strongly skewed in respect to the availability of these categories of trees in the study area (Lutermann et al., 2010). Second, intersex differences in gray mouse lemur were found and it has been proposed that females may control the high-quality sleeping sites, while males, due to the limitation of this resource, use low-quality sleeping sites (Radespiel et al., 1998, 2003). Third, sleeping holes are often used for several years and antagonistic interactions have been reported between females from a group and other individuals in front of sleeping sites (Dammhahn and Kappeler, 2009). Therefore, sleeping sites may represent a limited resource and the choice of the sleeping site can have a substantial effect on individual fitness.

Parasitism and the sharing of sleeping site

While sharing the sleeping site with conspecifics could bring several benefits, it could also come with a cost of increased risk of parasite transmission (Alexander, 1974). One species is often infested by a wide range of parasites that differ in their level of virulence and exhibit an impressive diversity of life history traits. Some of them are generalists, infecting a wide range of species, while others are specific to one or few animal species; some develop and reproduce within a same host, sometimes requiring a period of maturation within the environment (direct life cycle parasites), while others need to undergo several developmental stages in different particular “intermediary hosts” of several species before reaching maturity in a “definitive” hosts (indirect life cycle parasites). A large variety of mode of transmission results from these different life-history traits. For instance, ectoparasites, i.e. parasites living on the body surface of the host such as ticks and mites, can be transmitted through social contact and through the environment if they are able to survive outside the host. Endoparasites, i.e. parasites living inside the host’s body such as gastrointestinal parasites, can be transmitted through contact or ingestion of the infectious stages in food, water or through the oro-fecal route (Kappeler et al., 2015), depending on their life-cycle. Gastro-intestinal parasites with an indirect life-cycle cannot be transmitted between individuals of the same species and are generally transmitted through ingestion of a parasitized intermediary host. On the other hand, gastro-intestinal parasites with a direct life-cycle are contagious parasites that can be transmitted between hosts

of the same species, through direct contact with a parasitized individual or through contact with a contaminated environment.

Gastrointestinal parasite infection is often viewed as a proxy of the health status in natural populations and can be evaluated in a non-invasive manner through count of eggs in the feces (Hämäläinen et al., 2015). The ectoparasite burden can be also easily assessed by visual inspection of the animal body in some species. Experimental as well as correlative studies conducted in the wild showed that infection with both ectoparasites and gastrointestinal parasites can delay the growth rate and decrease body mass with tangible consequences on subsequent life history traits such as reproductive success or survival rate (Pedersen and Fenton, 2015).

The link between parasite transmission and sociality may appear obvious for contagious parasites and not evident at first glance for environmentally transmitted parasites. One may think that solitary and group-leaving species are exposed equally to those environmental sources. However, the probability of infection through a contaminated environment can be influenced also by social factors (Kappeler et al., 2015). For example, the degree of home range overlapping with conspecifics, as well as accumulation of feces in the habitat may increase the parasitism risk for group-leaving species. Indeed, using extensively a same area represent a risk in term of parasitism especially for gastrointestinal parasites as the environment will become more and more contaminated. In line with this, territorial and gregarious African bovids harbored more parasites than non-territorial ones (Ezenwa, 2004), probably due to the accumulation of fecal material in the environment. Surprisingly, in captivity, we observed that mouse lemurs defecate every day in their artificial sleeping sites. However, in natural holes the presence of fecal material is difficult to assess because the bottom of the holes is often hardly accessible. We observed that artificial sleeping sites placed in the forest also contained fecal material from mouse lemurs.

As the amount of fecal contamination in the environment should be correlated with group size, the risk of transmission with environmentally-transmitted parasites should be higher in larger groups. Individuals could minimize the risk of transmission by deserting contaminated environment or nests. In primates, mangabey (*Cercocebus albigena*) apparently move longer distances and use larger areas in response to more contaminated environment (Freeland, 1980). Yellow baboon (*Papio cynocephalus)* groups alternate between several sleeping sites: they leave a site after 1-2 days of use and avoid reusing it for an average of 45 nights, which could serve as a parasite avoidance strategy (Hausfater and Meade, 1982; Markham et al., 2016). Moreover, different species of bird and mammals have been found to prefer non-parasitized nests and desert nests or habitats when contaminated (Loye, J. Carroll, 1998; Reckardt and Kerth, 2007).

Interest and objectives of the study

So far, sleeping site ecology and parasitism have been compared in gray mouse lemurs only between sexes, but never between solitary and group sleeping females. For this study I took advantage of the unique facultative sociality in mouse lemur that allows us to directly compare solitary and group-sleeping females. The main goal of the study was to investigate the sleeping site ecology and the level of parasitism in the light of benefits and costs of sociality.

I aimed to answer three main questions:

1) Is there competition between females for sleeping sites? If yes, do group-sleeping females benefit from better quality sleeping sites?

To answer this question, collared-females were radio-tracked during day time to localize their sleeping sites and collect information on the usage patterns of sleeping sites. Characteristics of the sleeping sites were also recorded. Under the hypothesis that group-sleeping females may defend better resources, I predicted that they should use sleeping sites of higher quality compared to solitary females, in term of thermoregulation and predation risk. Therefore, I expected that group-sleeping females would use sleeping sites located higher in the trees, in larger trees and more often in living trees. As a result of having better quality sleeping sites, group-sleeping females may change sleeping sites less often than solitary ones.

2) Do group-sleeping females suffer from a higher parasitism level?

To test if sharing the nest brings a cost in terms of parasite infection, females were briefly captured to count the ectoparasites present on their ears, and to collect fecal samples to assess their gastrointestinal parasite status.

2a) Does parasite infection represent a cost in the gray mouse lemur?

To this aim, I investigated if ectoparasites and gastrointestinal parasites have an effect on the weight and on the probability of survival of individuals.

2b) Are group-sleeping females more parasitized than solitary females? Is parasitism related to group size?

I focused for this question on both ectoparasites and gastro-intestinal parasites. For the ectoparasites and gastro-intestinal parasites with a direct life-cycle (transmitted between hosts), I predicted that group-sleeping females should be more infested. However, if group-sleeping females exhibit some parasite avoidance strategies such as sleeping-site alternation or grooming, they could be less parasitized than solitary females. For gastro-intestinal with an indirect life-cycle, I predicted no differences between group-

sleeping and solitary females, as transmission occurs through the ingestion of a parasitized intermediary host.

3) Do female mouse lemurs show particular patterns of sleeping site occupancy that could act as a behavioral parasite avoidance strategy?

Under the hypothesis that gray mouse lemurs defecate in their sleeping sites, group-living as well as solitary females may have adopted some particular movement patterns to minimize their exposure to parasites in contaminated sleeping sites. In particular, I predicted that both solitary and group-sleeping females should avoid returning to recently used sleeping sites. Moreover, group-sleeping females may change sleeping sites more often than solitary ones to minimize their exposure to parasites, as they should contaminate more quickly tree holes, than solitary females.

METHODS

Study site

The study was conducted in the deciduous secondary forest of Kirindy in Western Madagascar. The site is located inside a forestry concession of the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie (CNFEREF) where the German Primate Center (DPZ) has established a field station in 1993. This area is characterized by high seasonality, with a rainy season lasting from December to March followed by a dry season of 7-8 months. In the dry season, both temperature and humidity strongly vary between day and night, with cold nights (reaching less than 4°) and hot days (reaching more than 32°). The study was performed using data collected during two field seasons: (1) in 2010, those data were collected by Elise Huchard during her post-doctoral position, and (2) in September-December 2016, during which I was on the field to collect additional data. These months correspond to the last months of the dry season and the starting of the mating season (estrus females where captured from beginning of October). The study was conducted within the core area of the study site, representing 9 ha. This area is free from logging activity since the establishment of the field station. It is adjacent to a small river and organized in a regular trail system with small foot trails every 25 m. Every intersection is marked with a tag allowing easy orientation.

Study population

Gray mouse lemurs, family Cheirogaleidae and suborder Strepsirrhini, are small (mean body mass 60g) primates. They are omnivoruous, feeding on fruits, insects, gum and insect secretions (Corbin and Schmid, 1995). Gray mouse lemurs can reach an age of 10 years in the wild, but the average life expectancy is much lower likely due to a high predation pressure: on average, males live 2.7 year and females live 3.4 years,

(Kraus et al., 2008; Hämäläinen et al., 2015). Whereas males stay active all year long, females can enter in hibernation during the austral winter, a period characterized by low food and high predation risk (Kraus et al., 2008). The mating period starts after females emerge from hibernation and lasts for four weeks between October and November (Eberle and Kappeler, 2006). However, the receptive period of each female lasts only one night, during which they promiscuously mate. The mating system is defined as multi-male/multi-female system (Radespiel, 2000). Females can give birth to one to three young after a period of gestation of two months (Eberle and Kappeler, 2006). Young develop quickly and start to mate in the first breeding season following birth. Males disperse before the reproductive period whereas females stay within or close to their mothers. Dispersion of the males has been shown to have a role in gene flow, which act as a mechanism to avoid inbreeding (Wimmer et al., 2002; Huchard et al., 2016).

The population of mouse lemurs has been studied since 1994: individuals are regularly trapped on a monthly basis, using Sherman life traps, and all individuals trapped for the first time are marked with subdermal a transponder. Moreover, sex, standard morphometric measures and reproductive status are recorded on each trapping session. Most individuals are captured in their first year of life and therefore the ages of all individuals can be accurately estimated.

Study subjects and determination of sleeping group composition

During the trapping sessions females that weighted more than 50 g were selected for our study, in an attempt to select adults. After anesthesia with 0.04 ml of Ketanest (100 mg/ml), the selected females were equipped with radio-collars (BD-2C weighting 2g, Holohil Ltd., lifespan of 14 weeks). A total of 22 females have been radio-collared in the 2010 field season. Additionally, 9 females were equipped in 2016. During both field seasons, females were radio-tracked during daytime to find their sleeping sites. Using a transponder reading device (Trovan reading system: maximum penetrable thickness of wood: 7 cm) placed near the tree hole, we were able to assess if the equipped females were alone in the nest, or if they shared the nest with other individuals. In the latter case, we determined the identity of each sleeping partner, by reading their microchip with the transponder. To reach the height of the sleeping sites with the transponder, an articulated device with tubes was built. The group composition and the localization of the nest used were determined between four and seven times per week. However in the 2016 field season, due to technical problems, the collars were removed before the end of the season, allowing data collection from for 40 days. In total, sleeping condition (solitary or group) were recorded for 30 individuals (26 females, 4 males) in 2010, and 17 individuals (16 females, 1 males) in 2016.

Sleeping site characteristics and usage

In 2016, we recorded data on the characteristics of the trees in which the sleeping sites were identified. In particular, trees were classified as dead or alive and the height of the sleeping site, by visual estimation, as well as the diameter at breast height (DBH) were recorded. Two variables were used to assess the sleeping site usage and fidelity. First, for each solitary female and each group, I calculated the average duration of occupancy (in days) of sleeping site, corresponding to the number of days of sleeping site observation (for each solitary female and group) divided by the total number of different sleeping sites used (for each solitary female and group). Second, to determine the usage patterns over time, I calculated "the return probability to the previous site" i.e. the probability that a female returned to the sleeping site used the previous day. To do so, I divided the number of days in which the solitary or sleeping-group females returned into the previous sleeping site by the total numbers of possible return days (two successive days when the sleeping site was successfully determined account for one possibility of return). Both measures have been already used in previous papers (Radespiel et al., 1998, 2003). For both variables (duration of occupancy and the return probability to the previous site) I always considered the group as an entity: when a female from a group left the group for one night, I considered the localization of the group. Data from both field seasons were included in the analyses.

Parasite sample collection and analysis

Parasite samples were collected in the field seasons 2010 and 2016, following the same protocol. Most of the samples were collected during monthly regular captures. In details, every month for three consecutive days, 160 Sherman traps were placed in the interceptions of the trails. The traps were set at dusk in trees, at a height of 1-2 meters, with a small piece of banana inside, and checked every following morning around 5:00 am. Mouse lemurs trapped were bought in the field station for the routine measurement. Fecal sample inside the traps were collected and stored in tubes with formol 10% until coproscopic analyses. Individuals were released in the localization where they were trapped the previous day. In 2010, 430 fecal samples were collected (see Table 1) of which 126 were from females whom sleeping habit was known. In 2016, in addition with the fecal samples collected during regular trapping sessions, I performed additional capture events to increase sample collection: after checking the location of the sleeping site during the day, I set traps in the sleeping site vicinity at dusk to increase the probability of capturing the females of interest. For this protocol, I checked the traps after two hours to avoid long confinement of individuals, and collected the feces when present. To avoid contamination of individuals and samples, all the traps were cleaned and disinfected before reuse. In total in 2016, I collected 59 fecal samples (see Table 1) of which 38 were from females whom sleeping habit was known.

In 2010, coproscopic analyses were performed at the Institut Pasteur of Antananarivo with the Ritchie's formol-ether concentration method that is a sedimentation method that allow to separate the parasites from fecal debris. Parasite eggs were assigned to the closest genus. In 2016, I performed coproscopic analyses following a flotation protocol in the lab in the field station of Kirindy (N = 42) and in the lab in the German Primate Center in Goettingen (N=17). Fecal samples were analyzed using a flotation protocol. This method is based on the high specific gravity of a saturated solution to float the lighter ova or cyst of gastrointestinal parasites. A solution saturated with sugar was used as flotation media. The procedure started with the stir and soften of feces with the flotation media. Then the mixture was filtered through a wire sieve and next, collected in a 5 mm tubes. At this point, a coverslip was placed on the tube. After 10 minutes were examined under the microscope. Flotation method is efficient to detect most of nematodes eggs. However, the quantity of the eggs found can depend on different factors like the flotation media and the waiting time before examination. Therefore, it is a valid method to perform qualitative analysis but not quantitative ones. Regardless, fecal egg counts method has been criticized for the inaccuracy as the rates of egg shedding can vary over time and it is not always be a proxy of the level of infection suffer by the individual. The variable parasite richness, i.e. the count of the number of different egg morphotypes, was calculated.

Moreover, during monthly trapping sessions or other occasional trapping events, ticks present on the ears of each individual were counted (no ticks have been seen on other parts of the body). In 2010, 289 tick counts (i.e., couting event) were recorded from 133 individuals (62 females and 71 males). Out of 289, 81 tick count were from females whom sleeping habit was known. In 2016, 214 tick counts were recorded from 86 individuals (37 females and 43 males). However, only 30 tick counts were from females whom sleeping habit was known.

Table 1. Sample size of fecal samples and tick counts collected.

	Total number	Individuals	Females	Males
Fecal samples 2010	430	149	54	85
Fecal samples 2016	59	31	18	13
Tick counts 2010	289	133	62	71
Tick counts 2016	214	86	37	43

Statistical analysis

1) Is there competition between females for sleeping sites? If yes, do group-sleeping females benefit from better quality sleeping sites?

First, I calculated the proportion of sleeping sites localized in living trees used by solitary and group sleeping females. As all the sleeping sites were not used at the same extent, I further calculated the proportion of days spent in living trees for both sleeping condition, and compared them with Fisher exact tests. Moreover, as solitary females alternate often between several sleeping sites (see Results), to investigate if they bias their choice toward one category of tree (i.e. in living or dead trees), I further performed an exact binomial test to compare the proportion of days spent in living trees with the proportion of "available" sleeping sites localized in living trees (i.e., used by solitary females).

Second, I compared the mean daily height of the sleeping sites and DBH of the trees between solitary and group sleeping females using the Mann Whitney U-test, as their distribution did not fit normality. To control for correlation between several characteristics of trees, I performed a correlation test between height and DBH of the sleeping sites, and a one-way Anova test to compare the mean height of the sleeping site and as well the DBH between alive and dead tree.

2) Do group-sleeping females suffer from a higher parasitism level?

In preliminary analyses, using generalized linear mixed models (GLMMs) with a poisson error distribution and logarithm link function, I investigated the influence of several determinants on parasite richness and number of ticks. I performed these analyses on the complete database including data from sexes and both seasons. In particular, I investigated the effect of the season (factor variable with 2 levels) and two individual characteristics, sex (factor variable) and age (continuous variable, in year). I further included as random factors the individual's identity to account for repeated sampling and the year.

2a) Does parasite infection represent a cost in mouse lemurs?

Using linear mixed model (LMMs) with a gaussian distribution, I investigated the influence of parasite richness and number of ticks on the weight of the individuals, controlling for environmental and individual characteristics (season/month, sex and age). As previously, individual's identity and the year were included as random effects. I conducted two LMMs, including either parasite richness or number of ticks, as these variables were measured with a different distribution during the year. Moreover, in the model with number of ticks, I included the effect of season instead of month, as ticks count was performed particularly from September to November and the sample size of the counts in the others months did not allow to test the effect of month. In the model with parasite richness as an explanatory variable, I included the effect of month (factor variable) and the interaction between month and sex as previous studies have shown that oscillation of weight throughout the year vary between males and females (Schmid, 1998).

To estimate whether parasite richness and number of ticks predicted intermediate term survival, I assessed the survival status of the individuals studied in 2010 after 6 months (binary variable: 0/1, corresponding to dead or alive individuals). I could assess the survival status of the individuals thanks to the monthly capture data. If an individual was never recaptured after that date (i.d. 31st of March or 31st of May) it was considered to be dead. Individuals that were captured only few times and lived at the edge of the core area, and especially males, were not included in the analysis as these individuals could have dispersed. Using generalized linear models (GLMs) with binomial error distribution and logit link function, I investigated the influence of the mean parasite richness and mean ticks count over the last 5 months on survival probability, controlling for the sex, the age the mean weight of individuals.

2b) Do group-sleeping females experience higher parasite infection? Is it related with group size?

I first compared the parasite status between group-sleeping females and solitary females. Males participating in the sleeping groups were excluded from the analysis to avoid bias given the higher parasite status of males (see results). Overall, 33 females from both seasons were included in the study, of which 23 were group-sleeping females and 10 were solitary. Moreover, both parasite richness and ticks count vary throughout the year. To account and avoid the effect of this variation throughout the year, I further restricted the analyses to the period from September to November, during which the information about the sleeping condition of females was the most important. To account for the different transmission route of gastrointestinal parasites, I analyzed separately parasite richness of direct life-cycle parasite and parasite richness of indirect life-cycle parasite. Using GLMMs with a poisson error distribution and a logarithm link function, I investigated if parasite richness or number of ticks differed between group-sleeping females and solitary females. I included in those models age and weight of individuals as control fixed variables. The year and the individual's identity nested within group (as individuals could belong to a common group and thus, they were not fully independent units) were included as random effects.

To further investigate the effect of group size on both parasite richness and number of ticks, I analyzed their variation within the group-sleeping females using GLMMs with a poisson error distribution and a logarithm link function. In these models, the mean group size (continuous variable) was considered. It was calculated as the sum of the number of individuals recorded in a group every day (females and males) divided by the total number of days, to have a more accurate measure of the group size. As previously, age and weight of individuals were included as control fixed variables, while the year and the individual's identity nested within group were included as random effects.

Multicollinearity was tested when multiple continuous dependent variables were included into the model. Age (in years) and weight (in gram) were found to be positively correlated. To correct for this correlation,

when both variables were included in a model, I substituted the weight measures with the residuals from a partial regression between weight and age.

For each analysis, the final model was selected based on lower Akaike Information Criterion values. When the response variables followed a poisson or binomial distribution, the model was tested for overdispersion. All the statistical analyses were done with the open source software R studio.

3) Does female mouse lemurs show particular patterns of sleeping site occupancy that could act as a behavioral parasite avoidance strategy?

First, I compared both duration of occupancy and the return probability to the previous site between solitary and group sleeping females using the Mann Whitney U-test. For solitary females, I further calculated the time elapsed between two successive occupancy bouts of a sleeping site (defined as "recursion time" in Hebblewhite, Merrill and McDermid, 2008), i.e., the number of days between the last day of one occupancy bout and the first day of the next occupancy bout. This recursion time was calculated even in the cases where there were days with missing information. Therefore, it could be likely overestimated. The recursion time of group-sleeping females could not be estimated over the course of the study as, due to the longer stay in a sleeping site (see Results) we never observed recursive events. We computed the mean recursion time considering 14 solitary females.

Table 2. Summary of the statistic tests performed for each question.

Title	Test
Characteristic of sleeping trees	Mann–Whitney test, Fisher's exact test
Usage pattern of sleeping trees	Mann–Whitney test
Determinants of parasite richness and tick numbers	GLMM
Survival analysis	GLM
Comparison of parasite richness and tick number between group-sleeping and solitary females	GLMM

RESULTS

For clarity sake, only the results concerning the variable of interests are given in this section. Full results of the models are given in supplementary tables (see Appendix).

Sleeping group composition

In 2010, 7 out 22 females were solitary. The others belonged to 7 different groups whit a mean size of 3.5 (range= 2-5, Table 3). One group included one juvenile male. One female slept alone for 9 nights and then

joined a group formed by other three males. In 2016, 5 out of 9 females were solitary. The others belonged to 4 different groups with a mean size of 3.66 individuals (range: 2-5, Table 3). However, one female that belonged to a group of 5 individuals for the first 14 days became solitary for the following 11 days. As in 2010, only one group included one juvenile male. The sleeping site of one female was to high (9 meters high) to be reached with the Trovan system. However, observation in front of this particular sleeping site at dusk, when individuals come out from the sleeping site, allowed us to categorize her as a group-sleeping female but the group size and the identity of the other group members remained unknown.

Table 3. Number of groups per each group size found in both 2010 and 2016.

Group size	N of groups in 2010	N of groups in 2016
1	7	5
2	3	1
3	1	-
4	1	1
5	2	1
NA	-	1

Groups size of 1 refers to solitary females. For one female, in 2016, the size of the sleeping-group could not be determined.

In both field seasons, we observed that some group-sleeping females slept alone some particular nights before returning sleeping in their group. Figure 1 shows the percentage of days at which all or part of the group members remained together.

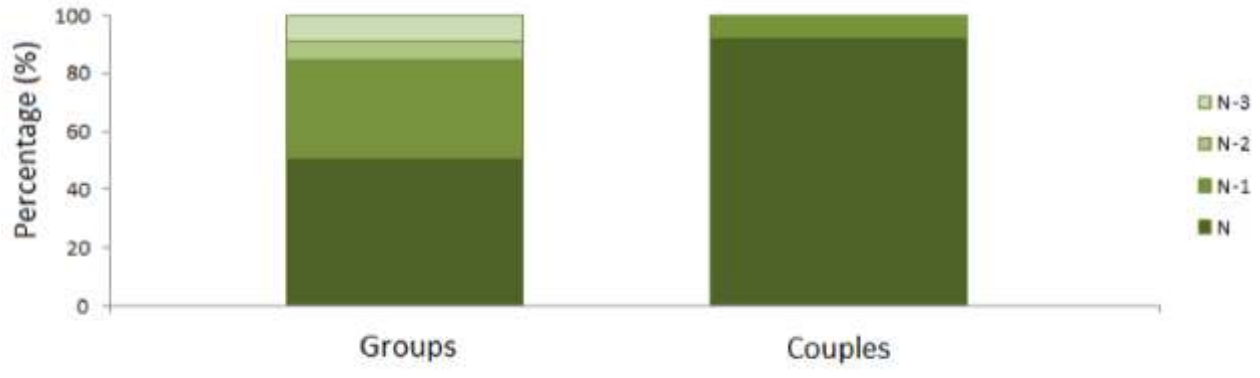

Figure 1. Percentage of days spent together in the sleeping site. N means that all the individuals observed in that group were together in the sleeping sites. N-1, N-2 and N-3 refer to cases where, respectively, one, two, or three individuals were missing in the group. I distinguished couples (N= 4) from groups with more than two individuals (N=6).

Sleeping site characteristics and usage

A total of 114 different sleeping sites were localized: 83 in 2010 and 34 in 2016. All these sleeping sites were tree holes except for one of them, located between the bark and the trunk, used one day by one solitary individual. The details of the sleeping sites found in 2016 are provided in Table 4.

Table 4. Average characteristics of sleeping sites used by group-sleeping and solitary females in 2016.

	Number of sleeping sites	Height (mean ± S.D.)	DBH (mean ± S.D.)	Percentage of living trees
Group-sleeping females	7	6.36 (± 2.05)	71.66 (± 41.82)	83.33 %
Solitary females	27	3.55 (± 2.29)	48.37 (± 23.97)	50%

The variable "height" refers to the height at which the sleeping sites were located and the DBH refers to the diameter at breast height of the tree were sleeping sites were located. Percentage of living trees were calculated as the number of living trees with a identified sleeping site respect to the total number of trees with identified sleeping sites.

We found that the mean daily characteristics of the sleeping sites in 2016 differed between solitary and group-sleeping females. First, group-sleeping females slept in tree holes significantly higher above ground (5.3m; range= 2–9.5 m) than solitary females (3 m; range= 0.2–8.5 m) (Mann–Whitney: N1=52, N2=101, W=4095, $P < 0.001$). Second, group-sleeping females slept in significantly larger trees (mean DBH = 61.94 cm, range= 23–152 cm) than solitary females (mean DBH= 42.69 cm; range= 18–96 cm) (Mann–Whitney: N1=51, N2=99, W=3455, $P < 0.001$). Third, group-sleeping females slept more often in living trees (82% of the days) compared to solitary females (37% of the days) (Fisher's exact test: $P<0.001$). Solitary females used 26 sleeping sites of which 13 in living trees and 13 in dead tree. Under random expectation, the percentage of days slept in both categories should be 50%. However, solitary females slept only 37.6% of days in living trees, which is statistically lower than the expected 50% that is the proportion of sleeping sites used by solitary female in living trees (exact binomial test $P<0.05$, 95% CI= 0.28-0.47). Finally, we found that the height of the sleeping sites and the DBH of the trees were positively correlated ($r^2=0.46$, $P<0.001$). However, neither the height nor the DBH were significantly different between alive and dead trees (Anova; $F_1=0.07$, $P>0.05$; $F_1=1.08$, $P>0.05$).

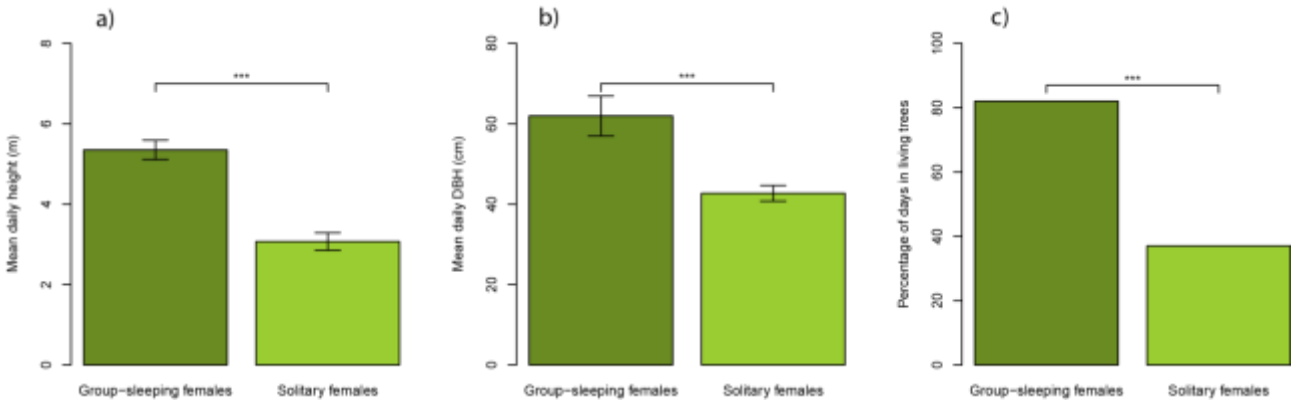

Figure 2. Differences of average daily characteristics of sleeping sites used by group-sleeping females vs. solitary females : a) height, b) tree diameter at breast height (DBH), and c) percentage of living trees . All values except for c) are mean ± SEM.

I had information about the sleeping condition on average 29 days per female in 2010 (range: 6-66 days). and 13 days per female in 2016 (range: 6-25 days). We found that group-sleeping females used more intensively the sleeping sites than solitary females: group-sleeping females showed an average duration of occupancy of 13.20 days/site (range: 5.17–26.00 days), whereas solitary females stayed to each site on an average of 4.20 days (range: 1.50–9.83 days) and this difference was statistically significant (Mann–Whitney: N1=11, N2=14, W=145.5, P < 0.001). Moreover, for group-sleeping females the return probability to the previous site was 0.98 (range: 0.82–1.00), whereas for solitary females this probability was 0.56 (range: 0.23–0.86). Again, this difference was statistically significant (Mann–Whitney: N1=11, N2=14, W=153, P < 0.001).

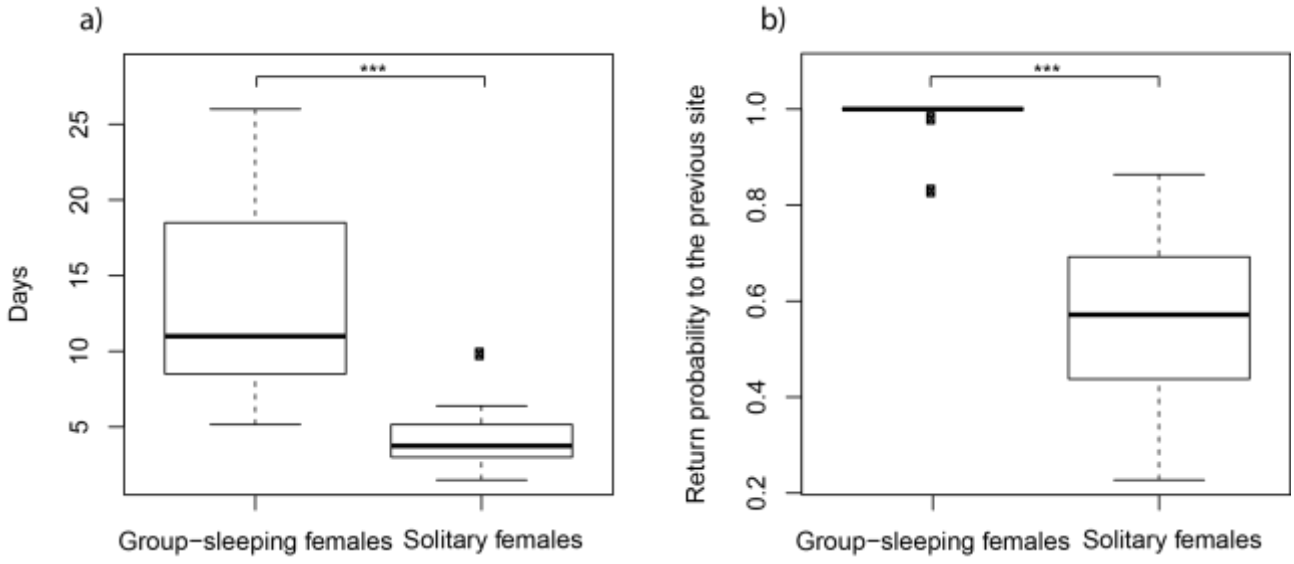

Figure 3. a) Duration of occupancy of the sleeping sites and b) return probability to the previous site for group-sleeping females (N= 11) and solitary females (N=14) (mean; box: standard error; whiskers: standard deviation (SD), ***: P<0.001)

Moreover, we observed 61 recursive events in 2010 and 17 in 2016 from solitary females. The mean recursion time for solitary females was 5.67 days (range: 2.00-18.00).

Parasite community and dynamics

In total, 10 different egg morphotypes were identified (Table 5). The average parasite richness was 0.96 ranging from 0 to 3 per sample (see Fig. 4a).

Table 5. Morphotypes and prevalence of gastrointestinal parasites found in *Microcebus murinus* in 2010 and 2016.

Genus	Prevalence in 2010 (%)	Prevalence in 2016 (%)	Transmission route
Ascaridae	0.22	15.25	Direct
Hymenolepis	23.15	40.68	Indirect
Metagonimus	0.22	0	Indirect
Oesophagostomum	0.45	0	Direct
Oxyuridae	8.09	16.95	Direct
Subulura	47.19	3.39	Indirect
Trichuris	16.63	5.08	Direct
SubuluraLarva	0.45	0	NA
Fasciolidae	0.67	0	Indirect
Coccidia	0.22	0	Direct
Lemuricola	-	10.17	Direct

Prevalence indicated as % of infected samples. The taxonomy, transmission routes were acquired from Hämäläinen et al., 2015.

Parasite richness was 1.41 times higher in males compare to females (GLMM, $z=2.84$, $P<0.01$) and 1.28 times higher in the wet season compare to the dry season (GLMM, $z=2.21$, $P<0.05$) (see suppl. Table 1). Moreover, individuals in 2016 showed a higher parasite richness compared to individuals in 2010 (GLMM, $z=1.93$, $P=0.05$).

In the complete dataset, the average number of ticks was 1.08 ranging from 0 to 12 ticks per individual (see Fig. 4b). Males carried 3.13 times more ticks compared to the females (GLMM, $z=4.79$, $P<0.001$) (see suppl. Table 2). The number of ticks was 2.63 times higher in the dry compared to the wet season (GLMM, $z=-4.46$, $P<0.001$).

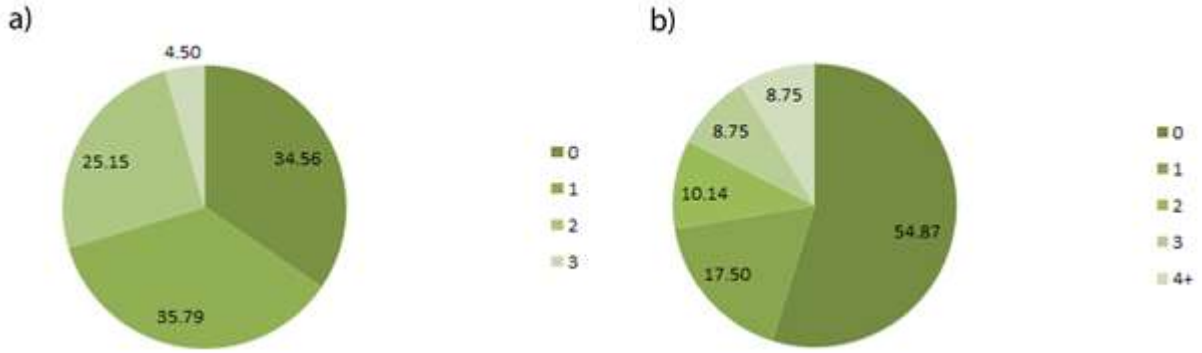

Figure 4. a) Repartition of fecal samples (%) according to the number of gastro-intestinal species found and b) repartition of tick counts (%) according to the number of ticks found.

Cost of parasitism

Parasite richness was positively correlated with weight (LMM, $P<0.05$) (see suppl. Table 3). Moreover, mean parasite richness was negatively correlated with survival after 6 months (GLM, $z= -2.12$, $P<0.05$) (see suppl. Table 5). On the contrary, the weight was negatively correlated with the number of ticks (LMM, $P<0.05$) (see suppl. Table 4) and the number of ticks did not affect the survival after 6 months (see suppl. Table 5).

Comparison of parasite infection between solitary and group-sleeping females

Neither the number of ticks nor the parasite richness, both of direct and indirect life-cycle parasites, differed between group-sleeping and solitary females (GLMMs, $P>0.05$) (see suppl. Table 6, Table 7 and Table 8). However, in the model including only data from the group-sleeping females I found that the mean group size was positively correlated with the number of ticks (GLMM, $z=2.36$, $P<0.05$) (see suppl. Table 9). Moreover, also parasite richness of direct life-cycle parasites was positively correlated with mean group size (GLMM, $z=2.43$, $P<0.05$) (see suppl. Table 10).Parasite richness of indirect life-cycle parasites did not vary with mean group size (GLMM, $P>0.05$) (see suppl. Table 11).

DISCUSSION

Sleeping group composition and stability

Groups were found to be stable over time: some females temporarily left the group (for one or few days) but rejoined it after a short period. However, in two cases we observed a switch between the two sleeping condition indicating that there might be some individual flexibility regarding the sleeping condition. First, in 2016, one female switched from sleeping with a group of 5 individuals (4 females and 1 male) to sleeping

alone. This event suggests that some females do not always sleep in group, even when relatives are present in the area. However, as sleeping groups during the dry season normally did not include more than four members, it might be that sleeping groups before birth season are limited in size (Radespiel et al., 2001). Similarly, it has been reported from another study site that a female used to sleep alone although a group of related females was in proximity (Radespiel et al., 2001). Second, in 2010, one female after a period sleeping alone joined a group formed by a couple of adult males, 2 additional males joined this group for two days. This report represents one of the few cases of adult males sharing a same sleeping site, and one of the rare cases of mixed-adult-sex group. While these reports are scarce, they provide some evidence that mouse lemurs may display plasticity in their sleeping condition.

Sleeping site ecology

The dry season in western Madagascar is characterized by a high daily fluctuation of temperature and a high predation pressure. Therefore, thermally insulated and safe sleeping sites were assumed to represent an important resource for mouse lemur females (Radespiel et al., 1998; Schmid, 1998). If the availability of high-quality sleeping sites within the environment has been high enough, we should have observed similar quality of holes between group-sleeping and solitary females. However, this study revealed a notable difference in sleeping site quality between group-sleeping and solitary females, suggesting that high-quality sleeping sites are a limited resource that individuals compete for.

Group-sleeping females slept in sleeping sites located on average in larger and more often living trees, and at a higher position above the ground. This result is in line with different studies conducted in deciduous forests during winter in different species of mouse lemurs, including the gray mouse lemur, that found that individuals preferred sleeping sites in trees with greater DBH that should therefore be more insulated (Schmid, 1998; Genin, 2010; Lutermann et al., 2010). Moreover, a higher position above the ground has been suggested to decrease predation risk. Therefore, the sleeping sites of group-sleeping females were on average safer and better insulated than those of solitary females, suggesting that group-sleeping females could be better able to compete than solitary ones for high-quality sleeping sites. However, two considerations have to be taken into account. The preference of group-sleeping females for tree holes situated in larger trees could be due to the fact that larger trees may have a higher probability to shelter larger holes compared to smaller ones. If this is true, it could also explain the difference in height of the sleeping site as the diameter at breast height was positively correlated with the height of the sleeping site. The status of the tree, alive or dead, was not correlated either with DBH or with height. Therefore, it appeared that group-sleeping females selected more often sleeping sites in living trees regardless of other characteristics. As a consequence of the limitation of this resource, solitary females could have less access to sleeping sites in living trees (representing 50% of the total sleeping sites used) compared to group-

sleeping females (representing 83.33% of the total sleeping sites used). Surprisingly, solitary females slept more often in the sleeping sites located in dead trees within the available sleeping sites, contrasting with the thermoregulatory hypothesis. Another study conducted on gray mouse lemurs also underlined a general preference of female gray mouse lemurs for sleeping sites in dead trees (Schmid, 1998). It has been proposed that this may be related to the fact that dead trees have a higher probability to shelter a hole compared to alive ones (Eppley et al., 2016). However, in this study it appeared that solitary females actually used more the dead trees than the living ones, among the sleeping sites they can use within their home range. A possible explanation is that solitary females may not really control all the sleeping sites where they have been localized. In line with this, in 2016, we found in five occasions another mouse lemur using a sleeping site of one of the solitary females of the study. Moreover, other species, including tenrecs, iguanids and tree boas have been found to use temporarily occupied the sleeping sites used by mouse lemur (Eberle and Kappeler, 2006). Therefore, the temporary occupancy of tree holes by other mouse lemurs or by individuals from other species may have forced them to use more often sleeping sites in dead trees. Further studies are needed to confirm that solitary females actively biases the choice towards dead trees. A possible experimental way to study both the preference for better insulated sleeping site and for sleeping sites situated higher in the tree could be to place artificial sleeping sites made by two different type of wood that differ in insulation capacity and locate them at two different height in the tree.

Solitary females used more sleeping sites and changed more often between them compared to group-sleeping females. It was suggested that this usage pattern could reflect a strategy to reduce predation risk: moving unpredictably between different sleeping sites might lower the probability of being detected by predators (Radespiel et al., 1998). If the sleeping sites of group-sleeping females are indeed more inaccessible, they can afford to stay longer in the same site. However, several other hypotheses could explain these different patterns of use between group-living females and solitary females. For instance, the coordination required between group-members to move from a sleeping site to another one may impose a constraint for group-sleeping females. On the contrary, solitary females can choose their sleeping site without this constraint, and therefore might choose every morning a sleeping site located in the foraging area explored the previous night. However, sleeping sites used by the same female/group were often located in proximity to each other, and solitary females were observed to forage close to different sleeping sites in the same night (Mosna and Socias Martinez unpublished data). Further investigations about the predation risks and the foraging area are needed to test these hypotheses. However, changing more often sleeping sites appears to be an intrinsic condition of solitary females. Interestingly, the female that have switched from a group-sleeping condition to a solitary one, have started to change sleeping nest with much higher frequency (from using 1 sleeping site for at least 14 days to change 4 sleeping sites within 9 days) when she became solitary. Accordingly, the same observation was made, but in the opposite direction, for the female that in 2010 joined a group after being solitary.

Overall, group-sleeping and solitary females showed several differences in the sleeping site ecology. It appears from this study that sleeping sites are a limited resource as previously suggested and that group-sleeping females are able to control sleeping sites of higher quality. Interestingly, solitary females shared several features in the sleeping site ecology with male mouse lemurs. Indeed, males and solitary females use sleeping sites located at lower altitude and more often in dead trees (Radespiel et al., 1998) than group-living females. Moreover, the probability of return in the previous site reported for gray mouse lemur males (0.44) (Radespiel et al., 2003) is similar to the one we found in solitary females (0.57), much lower compared to the one of group-sleeping females (0.98). Therefore, these results suggest that a distinction between solitary, including females and males, and group-sleeping females, is needed instead of the typical distinction between sexes when the sleeping site ecology or topics related to it are studied in gray mouse lemurs.

Parasitism

We first found, as a previous study on the same population (Hämäläinen et al., 2015), that gastro-intestinal parasite richness was higher in the wet than in the dry season, which may be explained by a better maturation or survival of parasites in humid conditions. In line with this, several previous studies have reported a higher prevalence or richness during wet season, in other host-parasite systems (Setchell et al., 2007). On the contrary, we found that individuals carried more ticks during the dry than the wet season. We further found that males harbored more ticks and gastro-intestinal species and ticks than females. This male biased parasitism has been reported in several mammals including in mouse lemurs (Mooring et al., 1996; Hämäläinen et al., 2015).

We performed two approaches to highlight a possible cost of gastro-intestinal parasites and ticks on individuals. First we used as a proxy individual's weight to see if more parasitized individuals were in worse condition than less parasitized individuals and second we investigated if parasites influenced the survival rate of individuals. We found that parasite richness was positively correlated with host's weight in the dry season, in accordance with recent findings (Hämäläinen et al., 2015). However, this positive relationship between parasite richness and weight might reflect that larger individuals, due to higher diet requirement, may be exposed to, and can internally accommodate larger numbers of parasites (Vitone et al., 2004). Furthermore, parasite richness negatively influenced the survival after six months suggesting that endoparasite infection brings a cost in terms of reduced survival in the long term. On the contrary, ticks had a negative effect on weight suggesting that tick infestation could bring a cost in terms of reduced energy. Accordingly, it has been shown that tick loads decreased the weight and body condition of hosts in several species (Jongejan and Uilenberg, 1994). However, tick infestation did not seem to influence the survival rate of individuals. The sample size of our analyses may be not adequate to answer this question as the

average number of ticks was calculated with an average of two tick counts per individual. For both gastro-intestinal parasites and ticks, additional studies and an experimental approach could help assessing the impact of parasitism on host survival and reproductive success.

Our prediction of increased parasitism level in group-sleeping females was not supported by the data. Neither richness of direct life-cycle parasites nor the number of ticks differed between solitary and group-sleeping females. However, both richness of direct life-cycle parasites and the number of ticks increased with mean group size when analyzing only groups. Interestingly, richness of indirect life-cycle parasites, transmitted by the ingestion of a parasitized intermediary host, was not influenced by mean group size within groups. Several hypotheses could explain those results, depending on the transmission route of these different parasites. For ectoparasites, individuals in small groups (2-3 females) may overcome a possible higher exposition to ticks than solitary individuals by grooming each other, masking a positive relationship between number of ticks and group size when including both solitary and group-sleeping females. On the contrary, larger groups could be exposed to a higher number of ticks than smaller groups and might not be able to overcome this cost by grooming, explaining a positive relationship between group size and number of ticks only in groups. Moreover, it's interesting to note that individuals in couples exhibited the highest fidelity to their sleeping partner, spending more than 90% of the days together (see Fig. 1). This high fidelity could suggest a closer relationship than the one between females in larger groups and could result in higher allogrooming activity.

For direct life-cycle parasites, group size could influence parasite richness if animals defecate in the sleeping site. Under this scenario, the amount of fecal contamination within a tree hole should be related to the size of the group using this hole, considering that different groups or different solitary individuals generally use different sleeping sites. Therefore, large groups may harbor more direct life cycle parasite species than small groups because they contaminate more their sleeping sites. The absence of a positive relationship between direct life cycle parasite richness and group size, when including also solitary females, could be explained by two different hypotheses. First, solitary females might use sleeping sites also used by other individuals, so the fecal contamination within the holes does not correspond to only one individual. Second, particular sleeping site usage pattern of solitary females may increase their exposure to parasites. Indeed, while group-sleeping females spent a period of approximatively two weeks before moving to another sleeping site, solitary females changed almost every few days, alternating between a fixed number of sleeping sites that are reused very often. Indeed, the recursion time for solitary females was 5.67 days (range: 2-18). The recursion time of group-sleeping females could not be estimated over the course of the study, as they returned in a previously used sleeping site after a long period. As a consequence the mean recursion time for group-sleeping females should be much higher than for solitary females. Even if more information is needed regarding the development and persistence of parasites within the tree holes, this

observed differences could explain in part the exposure to certain type of parasites. Indeed, among direct life cycle found in mouse lemurs, most of them are nematodes with a “long life-cycle”: when emitted in feces, nematode eggs need to undergo a period of development, lasting from few days to two-three weeks in the environment before becoming infective for other individuals (Neveu-Lemaire, 1952). Therefore, group-sleeping females might leave a sleeping site when nematode eggs within the sleeping site have reached the infective stage, and further avoid the sleeping site for a period of time long enough to allow some decontamination process to occur. On the contrary, as solitary females keep reusing the same nests continuously, they should be exposed continuously to infective stages of nematodes, explaining that there are not less parasitized than group sleeping females, even if the fecal contamination of their sleeping holes is expected to be lower.

In conclusion, these results suggest that females living in small groups might suffer the less from parasite infection, for both ecto- and endoparasites, compared to females sleeping in larger groups. We hypothesized that in mouse lemurs group sizes of 2 or 3 individuals might be optimal in terms of parasite burden, possibly due by particular sleeping site usage patterns and time spent grooming.

However, these results should be interpreted with caution due to some limitations of the study. First, an average of 3.36 fecal samples and 3.17 tick counts per individual were available. Therefore, further investigations are needed to test these hypotheses, with a higher sampling resolution to conduct fine grained analyses, especially considering that the number of nematode eggs emitted in fecal material can vary throughout the course of a parasite infection. Moreover, for some group sizes, we had just one or two groups of this size. As groups are kin based, individuals of one group may suffer from higher or lower parasite infection because of a higher or lower genetic susceptibility. Therefore, differences of parasite richness between groups found in this study could be explained by different susceptibilities to parasite infections rather than by the effect of group size. Furthermore, it would be interesting to correlate the parasite infection status with the dynamics of changing sleeping sites and study if the usage pattern of sleeping sites within groups is correlated with group size. This analysis could reveal if sleeping site usage patterns in mouse lemurs could be regarded also as a parasite avoidance strategy.

Benefits and cost of group-sleeping in gray mouse lemur

Females form sleeping groups whenever close female relatives are available, suggesting an adaptive significance of grouping (Radespiel et al., 2001; Eberle and Kappeler, 2006). Group formation can be driven by ecological pressures or by kin selection (West Eberhard, 1975; Wrangham, 1980). Food resources and sleeping sites are regarded as important ecological determinants of female association patterns (Kappeler, 1998; Schmid and Kappeler, 1998).

An previous experimental study have found that mouse lemur females adjusted their home range size with the availability of food resources (Dammhahn and Kappeler, 2009). An increased number of individuals exploiting the same area leads to a pauperization of the resources and therefore to a competitive regime between individuals. Foraging-related aggression or displacement were not observed frequently. However, in some occasions individuals were observed to displace others from high-quality resource patches and these aggressive interactions were mostly addressed to individuals that were not members of the sleeping group (Dammhahn and Kappeler, 2009). This study indicates that mouse lemur females may experience between-group competition over food resources, and that females may therefore form groups to access food resources. Our study further revealed that individuals also compete for sleeping sites in mouse lemurs, and that females may form groups to access better quality sleeping sites. The effect of competition over both ecological resources, food and sleeping sites, may account, therefore, for the female association pattern in this species. However, further studies are needed to compare the feeding time and the foraging range between solitary and group-sleeping females to confirm that group-sleeping females benefit from better quality food patches.

However, group-living has been associated to several costs, such as competition for food resources between individuals of a same group or higher parasite exposure. Interestingly, within group competition for food resources was found to be weak in mouse lemurs (Dammhahn and Kappeler, 2009). Moreover, we found that group sleeping females were not more parasitized than solitary ones, either because they are not exposed to a higher parasite pressure, or because they invest more in the immune system or have evolved some particular behavioral strategies that might counteract a higher parasite exposure.

Communal breeding could also represent in gray mouse lemur the main advantage of the group association. Sleeping groups have been proved to work as breeding units. In fact, females breed cooperatively taking care of all the group's offspring despite the ability to discern their own offspring (Eberle and Kappeler, 2006). This allonursing behavior has been proposed to be highly advantageous as it also provides the opportunity of adoption of closely related young, ultimately increasing inclusive fitness. The adoption of kin could be of particular benefit in mouse lemurs as juveniles experience a very high mortality rate, averaging 58% in a study (Eberle and Kappeler, 2006).

Kin selection might have been a stronger selective pressure compared to ecological pressures driving the group association in gray mouse lemur females. This hypothesis might be supported by the fact that, if the ecological advantages are high, solitary females with no relatives should form groups with unrelated females, but co-sleeping between unrelated females have never been reported (Radespiel et al., 2001). However, in 2010 for one night, two solitary females slept in the same sleeping site but they split the day after. Three explanations have been proposed to explain why unrelated females do not group despite the possible benefits (Radespiel et al., 2001). The first explanation states that the absence of close kin may be a

rare situation. However, as already noticed, this is not the case. Out of 31 collared-females in our study, 12 (38.7%) were solitary. Second, the difference in reproductive success between group-sleeping females and solitary females might not be large enough to act as a selective pressure. Solitary females might have evolved different behaviors to cope with the ecological pressure. For example, changing more often sleeping sites might be a good strategy to cope with predation risk. Third, affiliative relationships based on familiarity might be necessary to benefit from association between females and this familiarity might be facilitated by the long-term association between mothers and daughters and in general between the kin that share the same sleeping site. Developing familiarity between unrelated individuals could be more difficult. I further propose as a fourth explanation that if cooperative breeding is advantageous only when occurring between kin, then the costs of group sleeping between unrelated individuals would overcome the ecological benefit that grouping offers. Parasitism infection, feeding ecology (feeding time and foraging time) and predation risk should be further studied to definitively assess the differences between group-sleeping and solitary females. Reproductive success and survival are ultimately needed to compare solitary and group-sleeping females. The ambitious challenge remaining is to study whether the reproductive and survival differences between solitary and group-sleeping females are due to cooperative breeding or differential access to resources such as sleeping site and food or a combination of the two.

Conclusions

The aim of this study was to characterize the sleeping sites of gray mouse lemur females and their usage in order to determine the degree and patterns of competition between group-sleeping and solitary females over this resource. We furthermore tested the hypothesis that group sleeping females harbored more parasites compared to solitary females. For the first time, we have compared group-sleeping females and solitary females in the sleeping site ecology and parasitism level. Our analysis suggested that group-sleeping females indeed benefit from high-quality sleeping site but do not suffer from an increased parasitism. However, both ticks number and parasite richness increased with group size indicating that larger group suffer more parasitism and therefore, an optimal group size to cope with parasitism might exist in gray mouse lemur. Species with facultative sociality such as gray mouse lemurs represent unique models to study the benefits and costs of group association that ultimately could reveal why and under witch circumstances solitary females associate in stable groups providing insights into the evolution of sociality in primates and the evolution of cooperative behavior.

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APPENDIX

Analysis of Deviance Tables. Significance codes: *** if < 0.001, **<0.01,*<0.05. Df: degrees of freedom, Pr(>Chisq): probability of Type I error. P-values are two-tailed.

Supplementary Table1) Determinants of parasite richness

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Weight	2.5431	1	0.110778	
Season	4.9226	1	0.026507	*
Sex	9.9812	1	0.001581	**
Age	0.0105	1	0.918325	
Year	5.1352	1	0.023445	*

Supplementary Table 2) Determinants of number of ticks.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Weight	11.9503	1	0.000546	***
Season	20.0315	1	7.62E-06	***
Sex	22.9817	1	1.64E-06	***
Age	0.0444	1	0.83302	

Supplementary Table 3) Determinants of weight. Model including parasite richness

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	40.5352	1	1.93E-10	***
Sex	2.7225	1	0.09894	.
Month	108.2062	8	< 2.2e-16	***
Parasite richness	4.146	1	0.04173	*
Sex:month	96.0785	8	< 2.2e-16	***

Supplementary Table 4) Determinants of weight. Model including number of ticks

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	14.6379	1	0.00013	***
Season	0.2171	1	0.64129	
Sex	0.2173	1	0.641096	
N of ticks	6.1214	1	0.013356	*

Analysis of Deviance Table. Significance codes: *** if < 0.001, **<0.01,*<0.05. Df: degrees of freedom, Pr(>Chisq): probability of Type I error.

Supplementary Table 5) Determinants of survival after 6 months.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Sex	3.3215	1	0.06838	.
N of ticks	0.0215	1	0.8834	
Parasite richness	5.5531	1	0.01845	*

Supplementary Table 6) Determinants of number of ticks when comparing group-sleeping and solitary females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Weight	7.5008	1	0.006167	**
Sleeping condition	0.0044	1	0.947319	

Supplementary Table 7) Determinants of parasite richness of direct life-cycle parasites when comparing group-sleeping and solitary females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	0.4073	1	0.52334	
Weight	5.0414	1	0.02475	*
Sleeping condition	0.008	1	0.92893	

Supplementary Table 8) Determinants of parasite richness of indirect life-cycle parasites when comparing group-sleeping and solitary females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	5.3315	1	0.02094	*
Weight	0.4611	1	0.49712	
Sleeping condition	0.2617	1	0.60895	

Supplementary Table 9) Determinants of number of ticks within group-sleeping females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Weight	11.363	1	0.000749	***
Mean group size	5.5721	1	0.018249	*

Supplementary Table 10) Determinants of parasite richness of direct life-cycle parasites within group-sleeping females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	3.98	1	0.04604	*
Weight	3.9709	1	0.04629	*
Mean group size	5.9218	1	0.01495	*

Supplementary Table 11) Determinants of parasite richness of indirect life-cycle parasites within group-sleeping females.

Variable	Chisq	Df	Pr(>Chisq)	Level of significance
Age	4.389	1	0.03617	*
Weight	0.8275	1	0.363	
Mean group size	1.0838	1	0.29784	