

Sex-specific reproductive strategies of redfronted  
lemurs (*Eulemur fulvus rufus*, Primates, Lemuridae)

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# GENERAL INTRODUCTION

## **Reproductive strategies and sexual conflict**

In animals both sexes pursue different reproductive strategies in order to maximize their lifetime reproductive success (Trivers 1972). Sex-specific reproductive strategies have traditionally been explained by the generally higher investment of females in rearing young than males, beginning with the differential energetic investment in the production of egg or sperm (Bateman 1948, Parker et al. 1972; but see Dewsbury 1982). In mammals, this difference is later magnified by the costs of internal gestation and lactation (Williams 1966; Oftedal 1984). Parental investment has thus been proposed to determine the operation of sexual selection: The sex, which invests more in rearing young will become the limiting factor for the sex that invests less, which consequently has to compete with members of its own sex for available mates (Trivers 1972). While parental investment theory predicts the direction of competition in species with predominately maternal care, its limitations become apparent if applied to the wide range of species with uniparental male care, such as the majority of teleost fishes, anurans, urodeles, some invertebrates and some birds, where sometime males and sometimes females compete for mates (Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992). Clutton-Brock and Parker (1992) therefore replaced the concept of primary parental investment with the potential reproductive rate of males and females. The sex with the lower potential reproductive rate will be less available for mating, resulting in a skewed operational sex ratio (OSR; the ratio of receptive females to fertile males; see Emlen and Oring 1977). The skew of the OSR finally determines the strength of sexual selection, as well as the identity of the competitive and choosy sex.

The traditional dichotomy of coy, choosy females and competitive, sexually indiscriminate males (Darwin 1871; Williams 1966) is an oversimplification of the situation found in the real world (Gowaty in press). Hrdy (1977) was among the first to challenge this view by her description of sexually ardent, promiscuous primate females, competing for males. On the other hand, choosiness may be the strategy of choice for males, as could be shown in experiments on mate preference in mice and *Drosophila*, where males who reproduced with females they did not prefer had offspring of significantly lower viability than males that reproduced with preferred females (review in Gowaty in press). Animals of both sexes adopt flexible mating tactics responding to the given situation, e.g. a behavioural switch from choosy to

indiscriminate mating will be caused by parameters which decrease survival, such as parasite load, predation risk or age (Gowaty in press). The traditional Darwinian mechanisms governing sexual selection - male competition and female choice - have consequently been extended by these two factors, female competition and male choice.

Smuts and Smuts (1993) added male sexual coercion as another mechanism of intersexual selection, arguing that it depends on variation in the ability of males to coerce females. The more successful a male is in coercing females, the higher his lifetime reproductive success. Stressing the active role of females, female counter-strategies against male coercion, e.g. paternity confusion through promiscuous mating, was incorporated as yet another mechanism of sexual selection (Hrdy 1977; 1979). The terminology of “strategy” and “counter-strategy” (sensu “control” - “resistance”, Gowaty in press) indicates the fundamental conflict of interests between the sexes. The observed social organization (“who lives with whom?” Kappeler and van Schaik 2002) of a species may then be seen partly as a result of competing reproductive strategies of the sexes. In the present thesis I investigate how sex-specific reproductive strategies relate to the unusual group composition found in redfronted lemurs (*Eulemur fulvus rufus*) by combining behavioral, demographic and endocrinological data.

Despite flexibility in sex roles, reproduction in female mammals is limited mainly by the ability to successfully rear their offspring, which in general is influenced by the availability of food and other crucial resources as well as predator pressure (Rubenstein and Wrangham 1986). Hence the potential reproductive rate of females is lower than that of males and the OSR is biased towards males. Females distribute themselves according to the distribution of the risks and resources in the environment, whereas males, reproductively limited mainly by their chance to fertilize females, respond to the distribution of females (Emlen and Oring 1977). If predation pressure is high, females will live in groups in order to improve predator detection and to decrease per capita risk of capture due to dilution and selfish herd effects (Alexander 1974; van Schaik 1983). At the same time, however, within-group competition for food and safe positions will increase (Janson 1988), indicating that group-living is a compromise of improved safety from predators and increased feeding competition (van Schaik 1989). The third organizing factor of social organization is sexual coercion, for example in the form of infanticide by males (van

Schaik 1996; Sterck et al. 1997). Infanticide has been postulated to be an adaptive male reproductive strategy (Hrdy 1979) and it occurs in a variety of mammalian taxa, particularly among carnivores, rodents and primates (reviewed in van Schaik 2000a; b). A female counter-strategy against infanticide is to live in groups with one (or more) protector males (van Schaik and Kappeler 1997) and it has been shown that males effectively protect infants against attacking males (Borries et al. 1999)

### **Number of males in groups**

Given the choice, group-living females should generally prefer to live with many males over living with a single male because they derive certain ecological and social benefits from living in multi-male groups. First, males in some species are better at detecting and repelling predators than females (van Schaik and van Noordwijk 1989; Baldellou and Henzi 1992) and may thus lower the overall risk of predation (van Schaik and Hörstermann 1994) apart from lowering per capita predation risk (Hamilton 1971). Secondly, females will only be able to exercise mate choice if more than one male is present. By mating with several males females can, for example, insure fertilization (Bateman 1948) or increase the genetic variability of their offspring (Brown 1997). Finally, comparative studies have shown that the risk of infanticide is often lower in multi-male groups than in single-male groups because single-male groups face a higher chance of group take-over and subsequent infanticide (Newton 1986; Robbins 1995; but see Borries and Koenig 2000). Males, on the other hand, should prefer to live in single-male groups, where they can monopolize reproduction, over membership in a multi-male group, where they presumably have to share paternity among resident males (Kappeler 1999a; 2000a).

Under certain circumstances there are deviations from these general patterns and it may be in the interest of a female to live in a single-male group as it may be advantageous for a male to live with many males. For example, in feral horses offspring survival is higher in groups with a single stallion, because male harassment in multi-male groups adversely affects female physical condition (Linklater et al. 1999). Alternatively males might choose to live in multi-male groups, if male group size is directly related to female group size, as for example in lions (Packer et al. 1988) and many primates (e.g. Andelmann 1986). Depending on how paternity is shared among co-resident males it could be the better, albeit riskier, strategy at least for the dominant male to live in a multi-male group with more potential mates than to

be the single male with only few females (e.g. Launhardt et al. 2001). Regardless of the respective conditions favouring one or the other strategy it is obvious that the fitness interests of males and females concerning who lives with whom often diverge, giving rise to a conflict of interests between the sexes. The aims of this thesis are (i) to evaluate in redfronted lemurs how females regulate group size (Chapter 1), (ii) how males react to the given situation (Chapter 2) and (iii) how females, and possibly males, benefit from a particular group composition (Chapter 3 & 4).

### **Determinants of group composition and male-male competition**

Variation in the number of males per group has been associated with the spatial and/or temporal distribution of receptive females (Emlen and Oring 1977). Primates are particularly well-suited to evaluate reproductive strategies of males and females in relation to group composition because primates have been thoroughly studied and exhibit a great diversity of social organizations (Smuts et al. 1987; Janson 1992). Andelmann (1986) postulated female group size as the crucial determinant of male monopolization potential. In cercopithecine primates, groups of less than six females are generally single-male, whereas groups of more than ten females are multi-male, at intermediate group sizes both single-male and multi-male groups occur. These results were supported by further studies (Altmann 1990; Mitani et al. 1996a).

In contrast, Ridley (1986) identified the length of the breeding season, i.e. the temporal clumping of receptive females, as the determining factor of male monopolization potential and, hence, the existence of single-male groups. In species with longer breeding seasons fewer females will be receptive at a given time and a single male will be more capable to monopolize a group of females. In a recent meta-analysis it was shown that these two factors, absolute number of females and temporal distribution of their receptive phases, are not mutually exclusive, and that both influence male monopolization potential (Nunn 1999). Females may therefore counteract male monopolization potential by forming large groups, synchronizing their fertile periods (Ims 1990), or as a third alternative they may even leave their group and migrate into a group with the optimal number of males (Sterck 1997; Steenbeek et al. 2000).

The effect of estrous synchrony on male monopolization potential has been tested in several primate species, often using seasonality to estimate the degree of

reproductive synchrony (Ridley 1986; Mitani et al. 1996a). This approach may be misleading, however, if the fertile periods of individual females are extremely short (Bogart et al. 1977; Hrdy and Whitten 1987). Short fertile periods allow females to be in estrus asynchronously despite extreme reproductive seasonality of less than two weeks (Pereira 1991). The degree of estrous synchrony can only be reliably evaluated by monitoring ovarian cyclicity and endocrine function of receptive females.

The ability of males to monopolize access to females will lead to differences in the relative intensities of pre- and postcopulatory competition (Kappeler 1997). If monopolization potential is high and the reproductive success of males relies mainly on precopulatory competition, i.e. physical combat, selection will focus on traits enhancing fighting ability, such as body size and weaponry (Harcourt et al. 1981; Plavcan and van Schaik 1992; Mitani et al. 1996b). If monopolization potential is low and females mate promiscuously, competition will occur postcopulatory favouring males that are successful in sperm competition, and thus with relatively large testes (Harcourt et al. 1981). This pattern of interspecific variation in sexual dimorphism and testis size has been documented across anthropoid primates (Harcourt et al. 1981; Harvey and Harcourt 1984; Harcourt et al. 1995). Males living in single-male groups exhibited the most pronounced dimorphism and relatively small testes, males living in multi-male groups, with promiscuously mating females showed pronounced sexual dimorphism and large testes and in pair-living species sexual dimorphism was absent and testes were small. If agonistic relationships among co-resident males lead to the formation of linear dominance hierarchies, a positive relationship between male rank and mating success generally emerges (Cowlshaw and Dunbar 1991; de Ruiter and van Hooff 1993). A correlation between rank and actual reproductive success, however, seems only to hold for non-seasonal breeders, once again indicating that breeding seasonality lowers monopolization potential, because more females are receptive at the same time (Paul 1997).

Another potential mechanism of postcopulatory competition among males living in multi-male groups is physiological suppression of testicular function. In a variety of species, high-ranking males exhibit elevated levels of testosterone compared to low-ranking ones, which has been interpreted as physiological suppression (Schilling et al. 1984; Wickings and Dixson 1992; Brockman et al. 1998;



Kraus et al. 1999; Maggioncalda et al. 1999). By suppressing testicular endocrine activity of rivals, dominant males may increase their own reproductive success (e.g. Keverne et al. 1982; Arnold and Dittami 1997), because androgens are essential for sperm production and influence sperm quality (Wickings et al. 1986). Androgen seem also to play a role in the expression of aggressive behavior during phases of social instability, such as challenges by a conspecific male over access to mates or during the establishment of a dominance hierarchy (“challenge hypothesis”; Wingfield et al. 1990).

### **Lemur idiosyncrasies**

The above described causes and consequences of variation in the number of males in primate groups have been generated and tested mainly on anthropoid primates, where most predictions derived from the theoretical framework of sexual selection theory were supported (Kappeler 2000b). In contrast, the lemurs of Madagascar deviate in morphological and demographic traits both from theoretical expectations as well as from the patterns found in anthropoid primates. Because all extant lemurs are the result of a single colonisation event more than 50 million years ago and evolved independently from anthropoids (Martin 1990; Yoder et al. 1996) they provide a unique opportunity to study convergence in social organization and mating system within the socioecological framework.

Gregarious lemurs live in relatively small groups with only few adult females (Kappeler and Heymann 1996) and highly variable adult sex ratios (Kappeler 2000c). Despite a lack of sexual dimorphism in body and canine size (Kappeler 1990; 1996), which in general is associated with a low degree of direct male-male competition (Harcourt et al. 1981), co-resident lemur males can typically be arranged along a dominance hierarchy (Pereira and Kappeler 1997; Kraus et al. 1999; Ostner and Kappeler 1999). In anthropoids these characteristics would inevitably lead to the formation of single-male groups (Andelman 1986; Pope 2000). Single-male groups, however, are never the modal grouping pattern in gregarious lemurs; instead groups are characterised by an on average even or male-biased adult sex ratio (Kappeler 2000c). Considering the theoretical framework outlined so far, one may ask several questions: “Why do males not monopolize females?” or “How do females prevent monopolization?” “How do males, which lack morphological traits associated with direct male competition, cope with co-resident competitors?” “What are the benefits

gained by females from several males in their groups?” “Is it also in the males’ interest to live with many males?” This study of redfronted lemurs aims to answer these questions.

Redfronted lemurs exhibit the idiosyncratic demographic and morphological lemur traits. They live in small groups of 5 – 12 individuals with an even or male-biased sex ratio (Sussman 1974; Overdorff 1998; Wimmer and Kappeler 2002) and lack sexual dimorphism in body and canine size (Kappeler 1990; 1996). Nevertheless, during the mating season males engage in aggressive interactions over access to fertile females (Overdorff 1998; Ostner and Kappeler 1999). In wild populations either functional one-male groups with a dominant male are found, and the frequencies of social and sexual interactions with all females are skewed towards this male (Ostner and Kappeler 1999) or evidence of clear dominance relations among co-resident males is lacking altogether (Overdorff 1998; Gerson 2000). Paternity analyses revealed that the reproductive success was strongly linked with dominance rank, because the dominant male sired most of the offspring (Wimmer and Kappeler 2002).

### **Contents of the thesis**

Given the small number of females per group characteristic for redfronted lemurs, either the prevalence of single-male groups or female estrous synchrony lowering male monopolization potential is expected. Despite the methodological advances in the non-invasive endocrine assessment of ovarian function the reproductive physiology of many primate species remains poorly understood. This is particularly the case for the strepsirrhine primates, for which information on the reproductive endocrinology is available for only a few species, two species of galago (*Otolemur crassicaudatus*, *O. garnetti*), two species of loris (*Nycticebus coucang*, *N. pygmaeus*) and six lemur species (*Microcebus murinus*, *Varecia variegata*, *Propithecus verreauxi*, *Lemur catta*, *Eulemur mongoz*, *Eulemur macaco*; reviewed in Curtis et al. 2000). Moreover, socio-endocrinological studies of wild strepsirrhine populations have been restricted to the sifaka, *Propithecus verreauxi* (Brockman and Whitten 1996; Brockman 1999) and mongoose lemur, *Eulemur mongoz* (Curtis et al. 2000). Data on female reproductive physiology, however, are essential to assess basic reproductive characteristics (e.g. cycle and gestation length) of wild lemurs and

provide the basis for studies of the proximate mechanisms underlying female reproductive strategies. In Chapter 1, I will investigate the applicability of non-invasive endocrine measurements for monitoring of ovarian function and reproductive status in wild redfronted lemur females. I will therefore characterize the pattern of hormone excretion during the mating season and should this method be feasible, I will analyze the degree of reproductive synchrony among co-resident females.

The lack of sexual dimorphism in body and canine size (Kappeler 1990; 1996), in combination with relatively large testes (Kappeler 1997), indicate that redfronted lemur males compete indirectly for fertilizations. The possibility to distinguish dominant from subordinate males and the frequent aggressive interactions between co-resident males (Ostner and Kappeler 1999) allow the investigation of the social modulation of androgen excretion in redfronted lemurs. The hypothesis that males compete by suppressing testicular activity in rivals will be tested in Chapter 2. In addition, I will evaluate whether challenging seasonal factors (e.g. mating season, birth season) and social factors (e.g. rank reversals, group encounters) influence androgen excretion in males, as proposed by the challenge hypothesis (Wingfield et al. 1990).

In Chapter 3, the focus turns from the evaluation of mechanisms associated with a high number of males within groups to possible benefits derived from this situation. It has been proposed that females gain from surplus males in terms of improved thermoregulation during cold periods (Morland 1993; Overdorff 1998). Madagascar's pronounced seasonality with low minimum temperatures (Sorg and Rohner 1996) and the combination of low basal metabolic rate and high body temperature (Daniels 1984; Müller 1985) led to the hypothesis that lemurs respond to cold stress by inactivity and social thermoregulation, i.e. resting in tight body contact with one or several conspecifics, in order to conserve energy (Morland 1993; Pereira et al. 1999). This hypothesis has not been tested so far. In Chapter 3 this possible benefit for females derived from a high number of males in their groups will be evaluated.

The observation that redfronted lemur males can be aligned in a dominance hierarchy (Ostner and Kappeler 1999) and that dominant males sire the majority of offspring (Wimmer and Kappeler 2002) imply that monopolization of access to several females is apparently possible on the behavioral, but not the demographic level. The question therefore arises why dominant males do not expell subordinate

males from groups, as it is the case in many primates (e.g Sommer 1994). In Chapter 4, I will investigate factors determining the unusual number of males, combining behavioral and demographic data from several groups. Specifically, demographic parameters, such as birth and mortality rates as well as migration patterns, that proximately lead to an even or male-biased adult sex ratio will be evaluated,. In a second step, I investigate the variation in adult sex ratio and the temporal distribution of fertile females in order to assess male monopolization potential. Finally, I analyze which benefits are gained by females and/or males from living in groups with a relatively high number of males.

Overall, this thesis aims to evaluate proximate and ultimate causes of the unusual sex ratio found in redfronted lemurs, which seem to contradict basic predictions of sexual selection theory. By doing so, this study will contribute a piece of knowledge, which may eventually help to understand the puzzle of lemur social evolution.

## CHAPTER 1:

Endocrine characterization of female reproductive status in wild  
redfronted lemurs (*Eulemur fulvus rufus*)

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## INTRODUCTION

The application of fecal hormone analysis to wild populations of primates has recently led to important new findings in the study of primate biology. In this respect, estrogen and progesterone measurements in females have provided information on basic reproductive parameters, such as cycle and gestation length (e.g. Strier and Ziegler 1997; Ziegler et al. 2000a), allowed insights into the physiological mechanisms underlying reproductive seasonality (Strier et al. 1999; Ziegler et al. 2000) and, by examining hormone-behavior interactions, could illuminate the proximate mechanisms underlying female reproductive strategies (e.g. Brockman and Whitten 1996; Heistermann et al. 2001).

Despite the advances in the application of non-invasive endocrine assessment to wild living primates, the reproductive physiology of many primate species remains poorly understood. This is particularly true for the strepsirrhine primates (lorises, lemurs and galagos), for which information on female reproductive endocrinology is available from only a few species (reviewed in Curtis et al. 2000). Moreover, socio-endocrinological studies of wild strepsirrhine populations studies have been restricted to the sifaka, *Propithecus verreauxi* (Brockman and Whitten 1996; Brockman 1999) and mongoose lemur, *Eulemur mongoz* (Curtis et al. 2000).

Given that these studies indicated considerable species differences in the pattern of female reproductive hormone excretion, more data on other wild lemur species are not only needed to extend our knowledge about lemur reproductive endocrinology in general, but also to assess the extent of species differences in hormonal patterns underlying ovarian function and pregnancy both within lemurs as well as in comparison to other primate taxa. Moreover, data on female reproductive physiology are essential to assess basic reproductive characteristics, such as cycle and gestation length in wild populations of lemurs.

In this study we, therefore, investigated the applicability of non-invasive fecal endocrine measurements for monitoring reproductive status in wild redfronted lemurs (*Eulemur fulvus rufus*). Redfronted lemurs exhibit a highly seasonal reproduction (Hrdy and Whitten 1987; sole exception the aye-aye, *Daubentonia madagascariensis*: Sterling 1994), and studies in captivity suggest that the species is seasonally polyestrous, with most conceptions occurring during the first estrus (Izard et al. 1993). Based on examinations of vaginal smears, a cycle length of about 30

days has been reported for captive-housed animals (Boskoff 1978), and gestation length was reported to last on average 121.5 days, after which females usually give birth to a single offspring (Izard et al. 1993).

In the wild, redfronted lemurs live in relatively small multimale-multifemale groups with on average even adult sex ratios (Overdorff 1998; Ostner and Kappeler in prep). Animals are about 2 kg and lack sexual dimorphism in body and canine size (Kappeler 1990; 1996). Behavioral observations have shown that while females mate polyandrously during the short mating season, they mate more frequently with the central, dominant male of the group (Ostner and Kappeler 1999; Ostner et al. 2002). Within the mating season each female's fertile period is restricted to 1 - 2 days (Izard et al. 1993). Although females may copulate over a period of several days to weeks, behavioral estrus is characterized by an increase in the frequency of copulations as well as female solicitations and male mateguarding behavior (Pereira and McGlynn 1997; Overdorff 1998; Ostner and Kappeler 1999). An additional indicator of the behavioral estrus are intense aggressive interactions between males. However, female redfronted lemurs show no visual signs of estrus and the behavioral estrus can easily be missed owing to the redfronted lemur's cathemeral activity rhythm, i.e. animals are active day and night (Overdorff and Rasmussen 1995; Donati et al. 1999).

This paper provides information on endocrine changes associated with ovarian function and pregnancy in wild redfronted lemurs. The specific aims were 1) to characterize the pattern of fecal estrogen and progestogen excretion during the mating season and to compare the results from the wild with samples collected from two females in captivity; 2) to describe the changes in estrogens and progestogens throughout complete gestation and determine the earliest possible time to confirm pregnancy, 3) to provide information on cycle and gestation length and 4) to examine whether the pattern of fecal estrogens reflected the sex of the fetus in utero as was reported to be the case for urinary estrogens in the ruffed lemur, *Varecia variegata* (Shideler et al. 1983).

## METHODS

### **Study site and animals**

The study was carried out in Kirindy Forest, a dry deciduous forest located in Western Madagascar within a forestry concession operated by the Centre Formation Professionnelle Forestière de Morondava (see Ganzhorn and Sorg 1996 for a detailed description of the forest). Data were collected from two habituated social groups (groups A and B) of redfronted lemurs living within a 60 ha study area. The study covers the period from May 1999 to June 2000, thus, including two mating seasons in May/June and one birth season in October. At the time of the study the two groups comprised two adult females and 4 - 5 adult males (group A) and three females and 3 - 4 males (group B), respectively. At the start of the study the youngest female (PAP) was 32 months old and nulliparous, while the other four females were at least five years of age and had already born offspring in the previous years.

A total of 840 fecal samples were collected from the five females of both groups (see below). In addition, behavioral observations (2118 hours) were carried out on all adult members of both groups. We used continuous focal animal protocols from dawn to dusk (Altmann 1974) recording sexual interactions as well as mate-guarding behavior by males. Sexual interactions included copulatory behavior, in which intromission and thrusts were definitely observed, and mounts, which lasted only a few seconds and no intromission occurred.

### **Sample collection and hormone analysis**

Fecal samples were collected over the entire study period, thus encompassing a complete annual reproductive cycle per female (samples of one female were collected from the mating season until the second month of lactation, when this female died as a result of an accident). Frequencies of sample collection varied according to season from every day (mating season: May - June) to every fifth day (non-mating season: July - April), yielding a total of 840 fecal samples (84 - 191 samples per female). The only deviation from this sampling routine concerned a female that conceived just outside the mating season and, hence, samples were not collected daily. All samples were collected in the morning, at the onset of one of the main activity bouts (Donati et al. 1999), immediately after defecation. Samples were placed in 8 ml absolute ethanol in which they were stored at ambient temperatures



(25 - 35°C) for on average 14 days. Afterwards, samples were brought to the nearest town, Morondava, where they were placed in a refrigerator at 4 - 7°C until shipment to the laboratory for hormone analysis. In addition to the collection of regular samples from the five study females, three samples each of two late pregnant females were collected at the end of the gestation period in 2001 in order to increase the sample size for the investigation on the relationship between fecal estrogen levels and fetal sex.

Prior to hormone measurements, samples were homogenized in their original ethanolic solvent by squashing with a metal stick and subsequently extracted twice by shaking overnight on a horizontal shaker using the procedure described by Ziegler et al. (2000a). Efficiency of the extraction process, determined by monitoring the recovery of <sup>3</sup>H-progesterone (40.000 cpm) added to a subset of samples prior to homogenization was  $75.7 \pm 6.8\%$  (n = 30). Following extraction, the remaining fecal pellets were dried in a vacuum oven at 50°C and the dry weight determined. All hormone values were expressed as mass/g dry weight.

Because a radiometabolism study to determine the major estrogen and progesterone metabolites in redfronted lemur feces could not be carried out, group-specific assays for the measurement of immunoreactive total estrogen and 5 $\alpha$ -reduced-20-oxo pregnanes were applied to fecal extracts. Both groups of hormones are known to represent quantitatively abundant fecal metabolites of estradiol and progesterone, respectively, in mammals, including primates (Schwarzenberger et al. 1996; Heistermann et al. 2001), and their measurement has been shown to accurately reflect female reproductive function in a number of species (e.g. Brockman and Whitten 1996; Fieß et al. 1999; Curtis et al. 2000; Heistermann et al. 2001). Furthermore, HPLC chromatography analysis performed on late gestation samples from two wild females according to the methods described by Fieß et al. (1999) confirmed the presence of substantial amounts of both groups of hormones in the feces of the study species (see Fig. 1), suggesting that their measurement would also be suitable for assessing endocrine status in the study females.

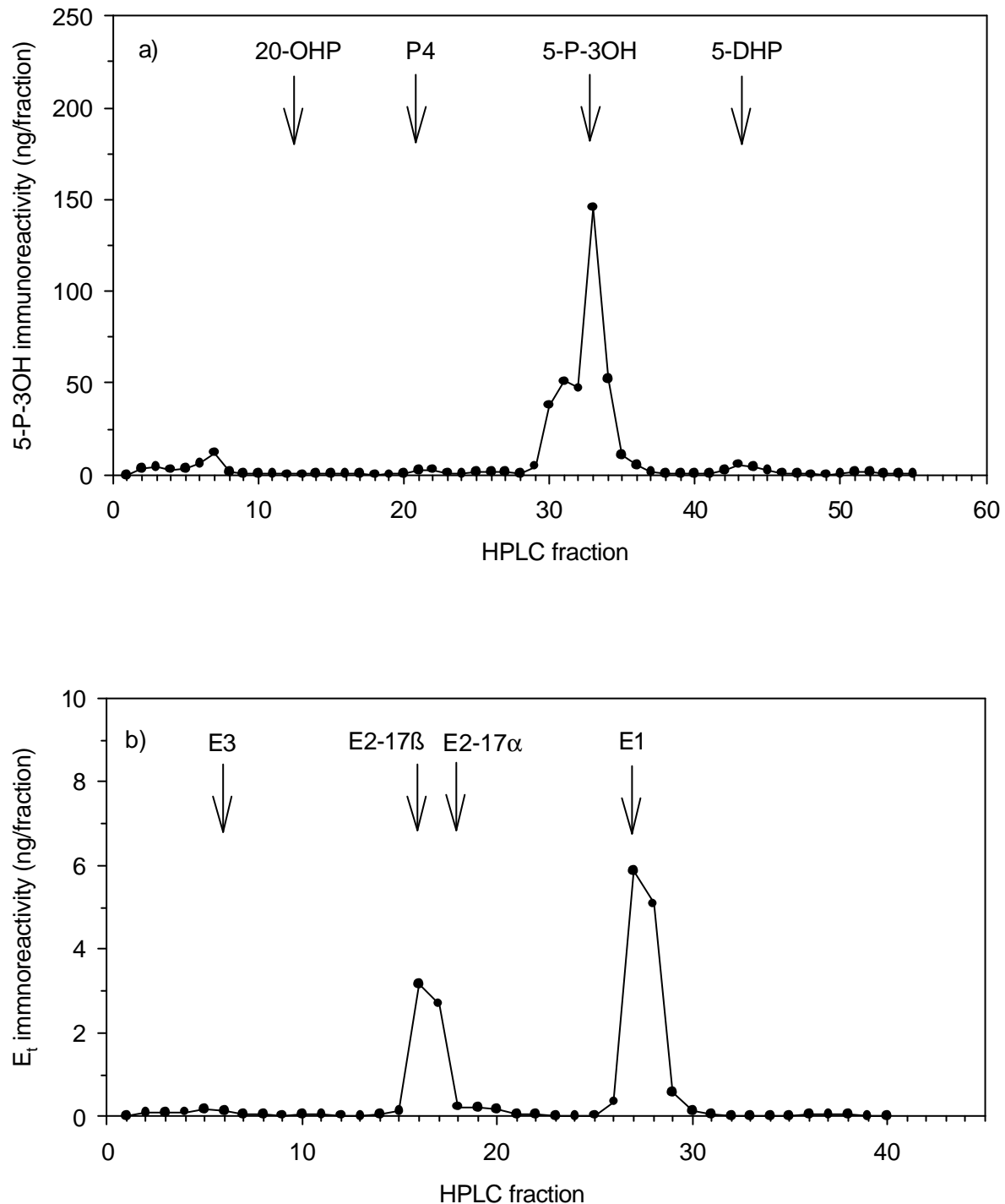


Figure 1: HPLC profiles of (a) 5-P-3OH and (b)  $E_1$  immunoreactivity in extracts of fecal samples collected from wild redfronted lemurs. Arrows indicate elution positions of  $20\alpha$ -hydroxyprogesterone (20-OHP), progesterone (P4),  $5\alpha$ -pregnane- $3\alpha$ -ol-20-one (5-p-3OH),  $5\alpha$ -dihydroprogesterone (5-DHP), estriol (E3), estradiol-17 $\beta$  (E2-17 $\beta$ ), estradiol-17 $\alpha$  (E2-17 $\alpha$ ) and estrone (E1) standards.

Immunoreactive  $E_1$  was determined using an antiserum raised in a rabbit against 1,3,5 (10)-oestratrien-3,17 $\beta$ -diol-17HS-BSA (Meyer et al. 1997) and estradiol-17 $\beta$  coupled to horse-radish-peroxidase (HRP) as a label. Fecal extracts were diluted 1:50 - 1:500 (depending on the reproductive status) in assay buffer (0.04

M PBS, pH 7.2) and 50  $\mu$ l aliquots taken in duplicate to assay. Unknowns and estradiol-17 $\beta$  standards (50  $\mu$ l, 1.9 - 250 pg per well) were combined with estradiol-HRP label (50  $\mu$ l) and antiserum (50  $\mu$ l), and incubated overnight at 4°C. Following incubation and washing of the plates, 150  $\mu$ l of HRP-substrate solution (3.125 mg tetramethylbenzidin in 250  $\mu$ l DMSO dissolved in 17 ml substrate buffer (0.05 M citric acid, containing 0.055 M Na<sub>2</sub>HPO<sub>4</sub> and 0.005 M hydrogen peroxide urea) was added to each well and the plates incubated on a shaker in the dark at ambient temperature for another 60 - 90 minutes. The enzyme reaction was stopped by adding 50  $\mu$ l 2 M H<sub>2</sub>SO<sub>4</sub> to each well and absorbance measured at 450 nm on a plate reader. Sensitivity of the assay at 90% binding was 1.5 pg. Serial dilutions of fecal extracts from samples of different females gave displacement curves parallel to that obtained with the estradiol standard. Intra- and interassay coefficients of variation, determined by replicate determinations of high- and low-value quality controls were 5.3% (n = 16) and 10.6% (n = 18) (high), and 8.8% (n = 16) and 14.6% (n = 18) (low), respectively.

5-P-3OH immunoreactivity in fecal extracts was determined with an EIA already described in detail by Hodges et al. (1997). Prior to assay, samples were diluted 1:40 to 1:400 in assay buffer. Sensitivity of the assay at 90% binding was 15 pg. Serial dilutions of fecal extracts from different females gave displacement curves parallel to that obtained with the 5-P-3OH standard. Intra- and interassay coefficients of variation, determined as described above were 6.4% (n = 16) and 9.2% (n = 18) (high), and 5.6% (n = 16) and 14.2% (n = 18) (low), respectively.

### **Samples from captive females**

Fecal samples were obtained from two captive females housed at Cologne Zoo, Germany, during the mating season from November 1999 until the end of January 2000 (n = 99 samples/female) for comparison. Although the animals were separated from their male group members for birth control reasons, they had visual, acoustic and olfactory contact to their group members

Daily fresh morning samples were collected immediately after defecation and frozen without preservatives at -20°C until analysis. Samples were lyophilized and pulverized using a pestel and mortar (Heistermann et al. 1993) and 0.05 g of the resulting dry powder was extracted with 3 ml 80% methanol in water by vortexing for 10 min (Heistermann et al. 1995). Following centrifugation, the supernatant was

decanted and after appropriate dilution in assay buffer (1:5 for  $E_t$ , 1:50 for 5-P-3OH) taken directly to the  $E_t$  and 5-P-3OH assay as described above. Efficiency of the extraction procedure, determined by monitoring the recovery of  $^3H$ -progesterone (40,000 cpm) added to a subset of the samples prior to homogenization, was  $95.1 \pm 3.3\%$  ( $n = 20$ ). Serial dilutions of fecal extracts from samples of both females ( $n = 4$  each) gave displacement curves parallel to that obtained with the estradiol and 5-P-3OH standard. Intraassay coefficients of variation for both assays were the same as described above. Interassay coefficients of variation of high and low value quality controls were 6.6% ( $n = 6$ ) (high) and 11.0% ( $n = 6$ ) (low) for  $E_t$  determinations and 10.2% ( $n = 6$ ) (high) and 13.7% ( $n = 6$ ) (low) for 5-P-3OH measurements.

## RESULTS

### Sexual behavior

The reproductive behavior within the two study groups was highly seasonal, in both years copulations were concentrated on the months of May and June. 92% of observed copulations ( $n = 182$ ) occurred within this period and dates of conception for each but one female (calculated by counting backwards from dates of subsequent births) also fell within this time period. For three of the five females it was possible to observe the day of behavioral estrus, which was characterized by male mate-guarding, intense male-male aggression, and female solicitations of copulations. One female showed a behavioral estrus about 35 days after her female group members and therefore well outside the usual mating season. All females conceived during the study and gave birth to a viable offspring. In four of the five females birth dates were clustered within a 10-day period between October 1<sup>st</sup> and 11<sup>th</sup>. The fifth female, which showed a behavioral estrus one month after the mating season gave birth about a month later (November 9<sup>th</sup>).

### Hormone profiles during the mating season

Representative profiles of fecal  $E_t$  and 5-P-3OH excretion during the mating season are shown in Figure 2 for two of the five study females in relation to periods of observed copulations, male mate-guarding and the day of behavioral estrus. Both hormones showed a high day-to-day variability over the entire mating season and no

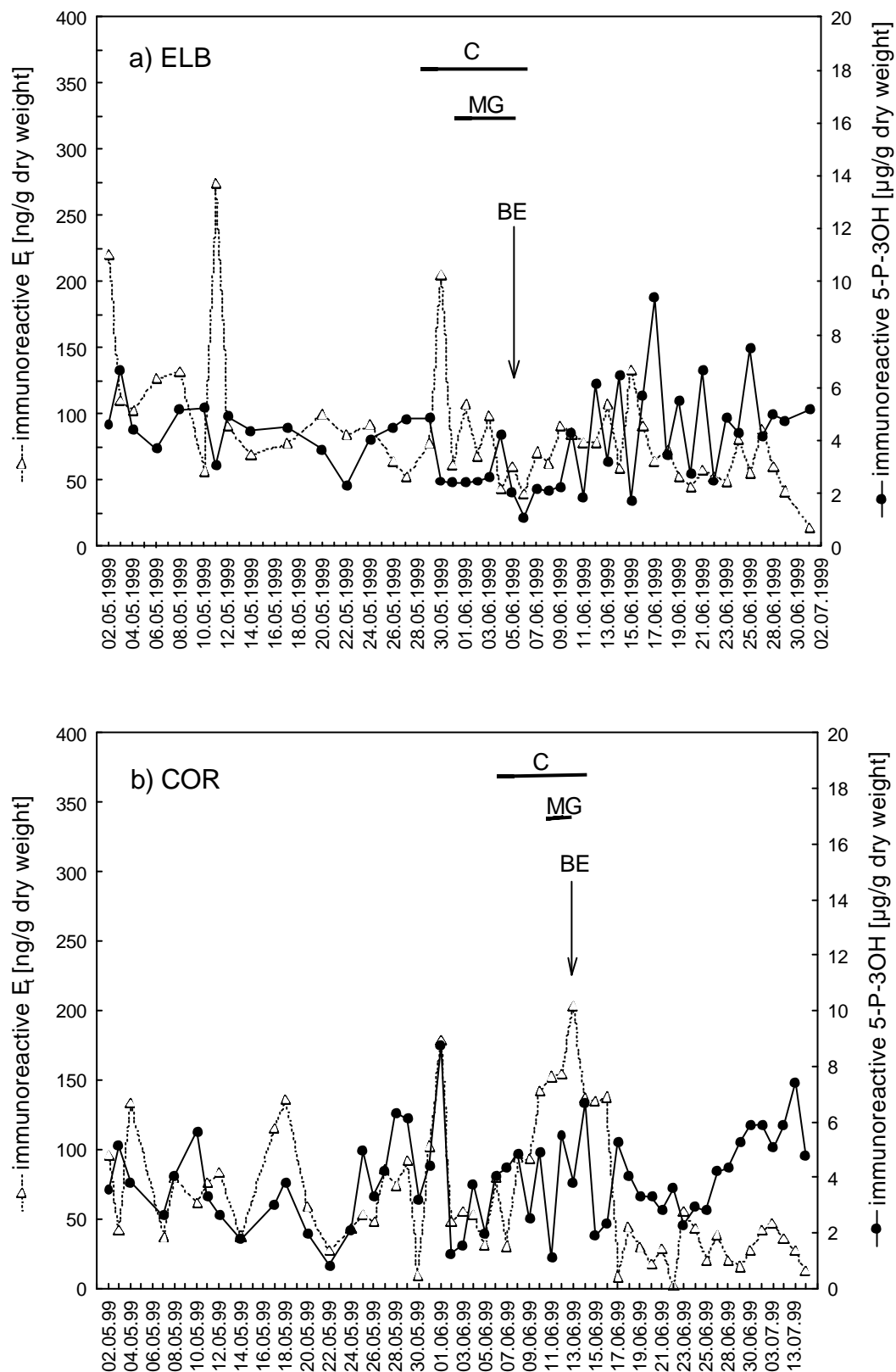


Figure 2: Individual profiles of fecal immunoreactive progesterone (5-P-3OH) and estrogen ( $E_t$ ) excretion of two wild redfronted lemur females during the mating season in relation to the occurrence of sexual behavior. BE: behavioral estrus; MG: mateguarding by central male; C: period during which the female was observed copulating. a) female ELB; b) female COR.

clear pattern from which the follicular and luteal components of the ovarian cycle could be distinguished. Although peaks in estrogen levels were seen they were not necessarily associated with periods of increased sexual activity and mate guarding behavior. Relatively low levels of 5-P-3OH during the period of estrus were in two cases followed by slight increases in levels thereafter (see Fig. 2a), but due to the high variability in levels, the onset of a post-ovulatory progesterone rise could not be deduced. Profiles were similar for the other three females.

Estrogen and progesterone profiles from the two captive females demonstrated also a considerable day-to-day variability (see Fig. 3 for one of the females as an example) but, in contrast to the females from the wild, 5-P-3OH excretion showed a clear cyclic pattern from which the component cycle phases could be discerned (Fig. 3). Based on successive 5-P-3OH rises, a mean cycle length of  $31.7 \pm 0.6$  days ( $n = 3$ ) could be deduced for the two captive females.

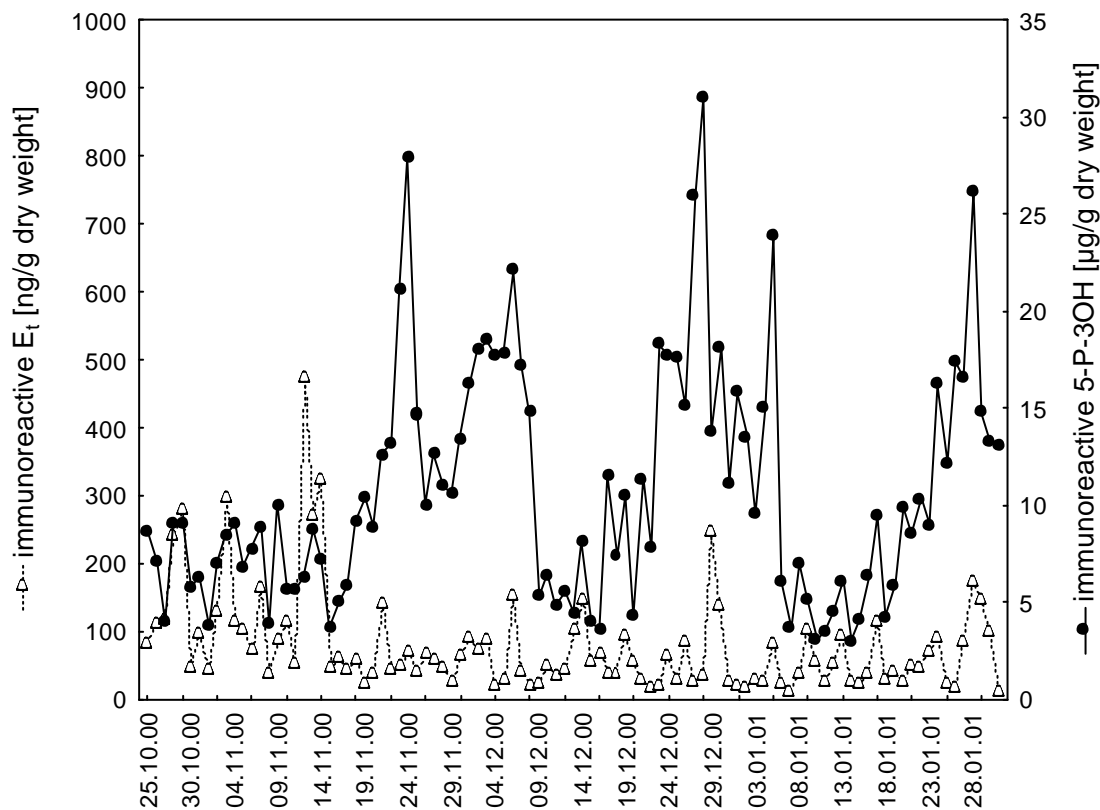


Figure 3: Profiles of fecal immunoreactive progesterone (5-P-3OH) and estrogen ( $E_t$ ) excretion of female 2417 from Cologne Zoo.

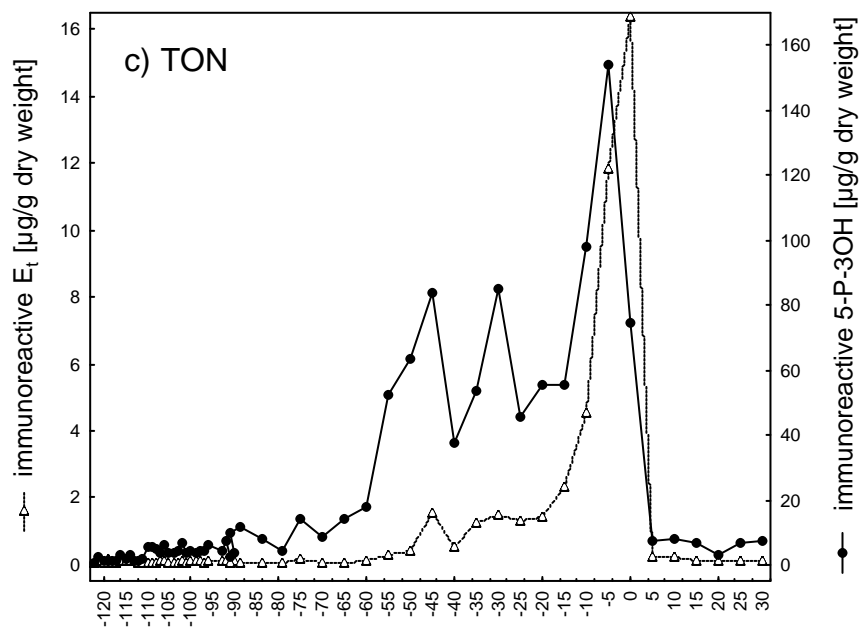
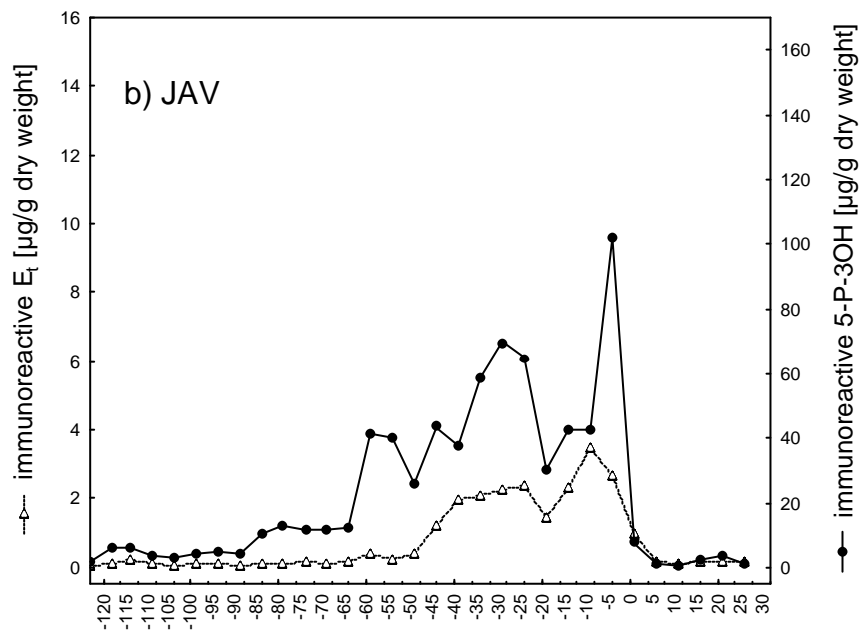
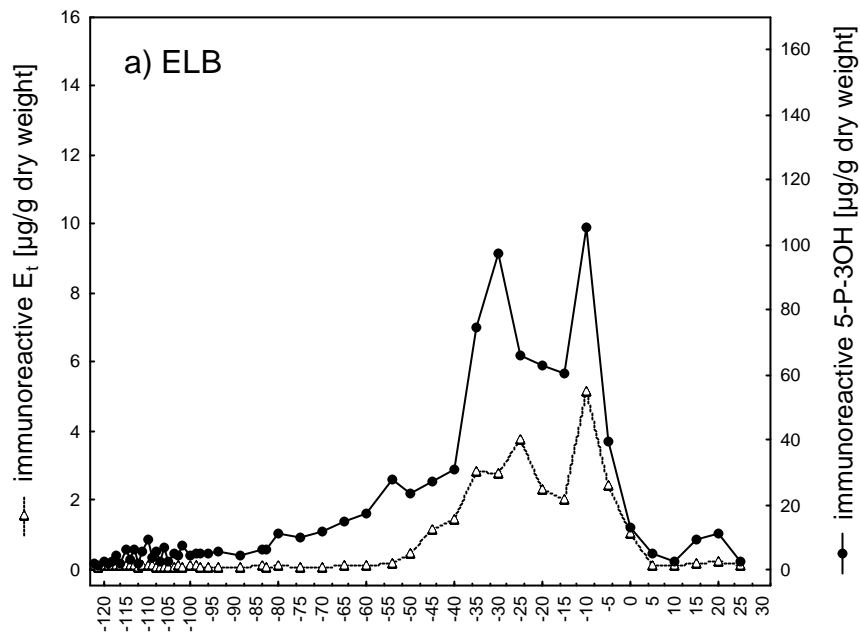
## Hormone profiles during pregnancy

Fecal estrogen and progestogen profiles throughout gestation in the five study females are shown in Figure 4. For the initial 40 - 45 days of gestation, 5-P-3OH levels remained at low mating season levels. Thereafter, in all females a gradual but sustained increase occurred, with the first significant rise (two standard deviations above the pre-conception baseline values) in 5-P-3OH concentrations being detected  $83 \pm 7.0$  days (range 94 - 75 days) before parturition. In four of the five females, highest concentrations of 5-P-3OH were found within the last five days before birth. Overall, levels of 5-P-3OH recorded during the last third of gestation showed a 15 - 35 fold increase compared to those from the mating season and early pregnancy (Table 1). Following parturition, levels declined to low mating season concentrations within five days.

The excretion of fecal immunoreactive estrogens followed a pattern similar to that of the progestogens in three of the five females (females ELB, JAV, TON, Fig. 4 a-c), except that the significant and sustained increase in levels was recorded about one month later ( $51 \pm 3.2$  days before birth). The levels were markedly (25 - 50 fold compared to mating season and early gestation) elevated during the last third of gestation (Table 1), with maximum concentrations being recorded in the week before parturition.

Table 1. Mean values (and standard deviation) of immunoreactive total estrogen and progestogen excretion from fecal samples of wild redfronted lemur females. The first three females were carrying male fetuses.

Female		E <sub>1</sub> [ng/g dry weight]				5-P-3OH [ $\mu$ g/g dry weight]			
		Mating season	1 <sup>st</sup> third gestation	2 <sup>nd</sup> third gestation	3 <sup>rd</sup> third gestation	Mating season	1 <sup>st</sup> third gestation	2 <sup>nd</sup> third gestation	3 <sup>rd</sup> third gestation
ELB	mean	105 $\pm$ 58	64 $\pm$ 20	387 $\pm$ 520	2768 $\pm$ 1240	3.8 $\pm$ 1.3	4.4 $\pm$ 2.0	19.3 $\pm$ 8.1	64.9 $\pm$ 29.8
	n	22	29	9	8	24	29	9	8
JAV	mean	87 $\pm$ 66	80 $\pm$ 42	573 $\pm$ 671	2338 $\pm$ 651	3.3 $\pm$ 2.3	6.3 $\pm$ 3.3	14.3 $\pm$ 28.0	102.2 $\pm$ 23.7
	n	4	9	8	7	31	9	8	7
TON	mean	87 $\pm$ 61	75 $\pm$ 25	302 $\pm$ 469	3789 $\pm$ 4096	3.5 $\pm$ 2.3	4.3 $\pm$ 2.5	29.7 $\pm$ 28.0	73.3 $\pm$ 35.9
	n	5	29	9	9	25	29	9	9
COR	mean	79 $\pm$ 42	73 $\pm$ 93	99 $\pm$ 40	162 $\pm$ 84	3.7 $\pm$ 1.7	4.6 $\pm$ 2.6	46.7 $\pm$ 28.7	96.6 $\pm$ 29.4
	n	32	27	8	7	34	27	8	7
PAP	mean	134 $\pm$ 66	117 $\pm$ 36	111 $\pm$ 34	98 $\pm$ 69	2.3 $\pm$ 1.0	4.9 $\pm$ 2.8	35.7 $\pm$ 18.1	79.6 $\pm$ 41.7
	n	25	29	9	7	25	29	9	7





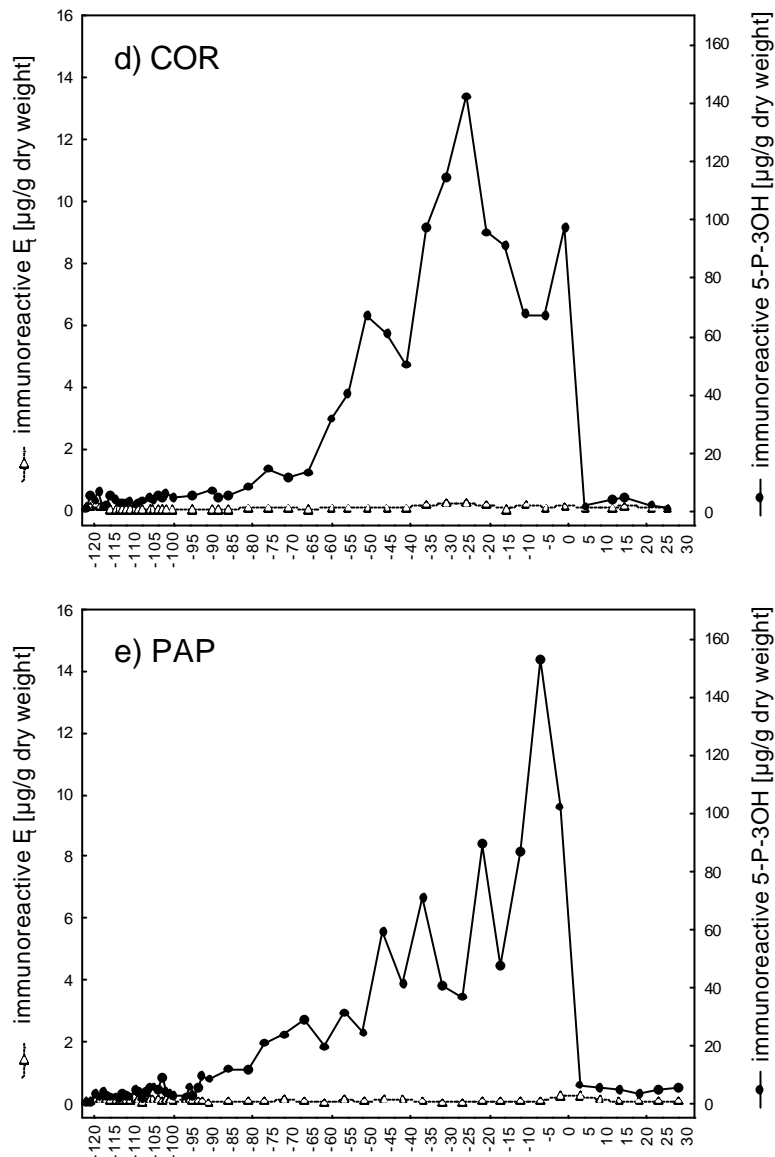


Figure 4: Profiles of fecal immunoreactive progestogen (5-P-3OH) and estrogen ( $E_t$ ) excretion during gestation in individual wild redfronted lemurs. Figures a) - c) represent females carrying a male fetus, while figures d) and e) represent females carrying a female fetus. Data are aligned to the day of parturition (day 0).

During the last week of pregnancy, levels gradually declined and reached the low mating season levels within five days of parturition. In two females (COR and PAP), estrogen levels remained at low mating season concentrations throughout the whole gestation period, with no clear elevation being observed (Fig. 4 d,e).

The two groups of females differed from each other with respect to the sex of the fetus they were carrying: the three females exhibiting a clear increase in estrogens during gestation gave birth to a male infant, while the two females showing consistently low fecal estrogen values gave birth to a female infant. A possible link between the sex of the fetus and the level of maternal estrogen excretion in feces

was re-evaluated using the three samples each of two females collected during the late gestation period in 2001. These females gave birth to a male and a female infant, and mean estrogen levels were  $966.0 \pm 188.0$  ng/g and  $64.4 \pm 45.2$  ng/g, respectively. Statistical comparison of fecal estrogen levels in male- versus female-carrying mothers showed that levels in the former were significantly higher than those in the latter (data from 1999 and 2001 combined: male-carrying:  $n = 4$ ; median = 1934 ng/g; range = 966 - 3024 ng/g; female-carrying:  $n = 3$ ; median = 109 ng/g; range = 64 - 140 ng/g; MWU-Test:  $U = 0.0$ ;  $p(\text{two-tailed}) < 0.05$ ).

Based on dates of behavioral estrus and birth, a mean gestation length of  $121.3 \pm 1.2$  days (122, 122 and 120 days) was calculated for the three females for which the day of behavioral estrus was known (see above).

## DISCUSSION

The present study provides the first account on the feasibility of fecal hormone analysis for monitoring ovarian function and pregnancy in wild redfronted lemurs. By providing information on the excretion patterns of fecal estrogens and progestogens throughout a complete annual reproductive cycle, we could generate the first data on the reproductive endocrinology of the species. By doing so, we show that fecal hormone analysis is a useful tool to monitor endocrine changes associated with pregnancy, while in our study it had certain limitations regarding the assessment of ovarian function.

Our HPLC results show that estradiol-17 $\beta$  and estrone were the major estrogens excreted by *Eulemur fulvus rufus*, with estrone predominating. Comparative information on the relative abundance of estrogens excreted into the feces of other lemur species is not available, but estrone is also the major estrogen excreted into the feces of two other non-lemur prosimian species, the slow loris, *Nycticebus coucang*, (Perez et al. 1988) and the pygmy loris, *Nycticebus pygmaeus*, (Jurke et al. 1998) and has also shown to be the predominating estrogen in the urine of the ruffed lemur, *Varecia variegata* (Shideler et al. 1983). HPLC co-chromatography also indicated that - as in other mammals (Schwarzenberger et al., 1996) - 5 $\alpha$ -reduced-20-oxo pregnanes represent abundant progesterone metabolites in the feces of redfronted lemurs. Since to date there is no comparative information on the metabolism and fecal excretion of progestogens for any other lemur species, it

remains to be shown whether the fecal excretion of high amounts of  $5\alpha$ -reduced-20-oxo pregnanes is a common phenomenon within lemurs.

Despite the fact that both groups of hormones were abundant in the feces of our study species, profiles did not show a clear cyclic pattern during the mating season and endocrine changes were not clearly related to sexual behavior and female attractivity, different to the situation found in *Propithecus verreauxi* (Brockman and Whitten 1996). A lack of a clear cyclic pattern in fecal estrogens and progestogens during the breeding season, however, has also been reported for wild female *Eulemur mongoz*, although in this species it appeared that, at least in some cases, sexual activity was related to elevated estrogen and low progestogen levels (Curtis et al. 2000).

The reason for the highly variable hormone profiles in the wild females is not clear, particularly since the profiles of the captive animals showed - at least for progestogen measurements - a clear cyclic pattern from which the component cycle phases could be distinguished. Whether this difference in steroid excretion patterns reflects real biological differences between wild and captive animals is not known, but appears to be unlikely. Two main factors, therefore, may have contributed to the higher variance in day-to-day hormone levels found in the wild females and to the different patterns observed. Firstly, compared to the captive animals females in the wild showed a much more pronounced and, in addition, highly variable excretion of undigested food. Since dietary fiber composition is known to affect fecal hormone concentrations (Wasser et al. 1993), it is likely that the high and variable proportion of undigested fecal matter in the feces of the females from the wild increased the variation in excreted hormones and resulted in the non-informative hormone profiles seen (c.f. Curtis et al. 2000). This assumption seems to be supported by the finding that in wild *P. verreauxi*, which excrete highly consistent "fecal droplets" (Ostner pers. observ.) the day-to-day variation in fecal hormones appears to be low, allowing ovarian function to be accurately monitored (Brockman and Whitten 1996). Secondly, since it has recently been shown that storage in ethanol for periods of more than a month can alter fecal hormone concentrations even when stored at lower temperatures (Khan et al. 2002), it is possible that the differences in fecal storage methods may have resulted in a higher variance and different absolute hormone concentrations in the samples from the wild. The kind of collection and storage methods used in this study, however, have been successfully applied to monitor

female ovarian function in a number of other studies (e.g. Wasser et al. 1991; Strier and Ziegler 1997; Ziegler et al. 2000a; Engelhardt et al. submitted ms) which may suggest that effects of storage methods and storage time on fecal hormone levels may depend on the species in question. Future studies are therefore needed to address the potential effects of diet and storage methods on fecal gonadal hormone concentrations in redfronted lemurs and probably other lemur species, particularly when the relatively subtle endocrine changes associated with cyclic ovarian function are being studied.

Unfortunately, it was not possible to determine cycle length in wild females. This may be difficult or impossible anyway, given that in most lemur species that exhibit a strict seasonal reproduction females generally conceive during the first breeding season cycle (e.g. Brockman and Whitten 1996; Curtis et al. 2000) as was probably also the case for the four study females that conceived in early June. The mean cycle length of 31.7 days determined from the progestogen profiles of the two captive animals, however, is in line with earlier data on cycle length of 30 and 28 days for this species as determined from changes in vaginal cytology (Boskoff 1978; Izard et al. 1993).

The hormone profiles generated during pregnancy indicated that fecal progestogen and estrogen measurements provide reliable information on the endocrine changes associated with gestation. The average calculated gestation length of 121.3 days found compares well with gestation lengths of 121.5 days and 122.7 days reported for captive animals (Izard et al. 1993; 1995). Pregnancy could be reliably determined after the first third of gestation (day 40 - 45) when, similar to the findings in *E. mongoz* (Curtis et al. 2000) and *Galago crassicaudatus* (Izard and Fail 1988), a significant increase in fecal progestogen levels occurred. The profile appears to differ, however, from that found in *P. verreauxi*, in which fecal progestogens have been reported to increase immediately following conception (Brockman and Whitten 1996). As in *G. crassicaudatus* (Izard and Fail 1988), but different to the situation found in *E. mongoz* (Curtis et al. 2000), in the redfronted lemur the rise in progestogens continued until the final week of gestation. The source of the elevation in progestogen levels after the first 6 - 7 weeks of pregnancy is not clear, but the increase is likely to be associated with a functional feto-placental unit (c.f. Butler 1960 for *Galago senegalensis*; Jurke et al. 1998 for *Nycticebus pygmaeus*). Because, to our knowledge, information on the onset of placental

steroidogenic activity is not available for any lemur species, this has to be confirmed in future studies.

Like excretion of progestogens, estrogen excretion remained low up to mid-pregnancy, thus deviating from the typical estrogen profiles seen in anthropoid primates, where gestation is usually associated with rising estrogen levels from about 2 - 3 weeks following conception (e.g. Hodgen et al. 1972; Czekala et al. 1983). A lack of an early pregnancy estrogen increase has also been reported for other prosimian primates (*E. mongoz*: Curtis et al. 2000; *P. verreauxi*: Brockman and Whitten 1996; *Varecia variegata*: Shideler et al, 1983; *N. pygmaeus*: Jurke et al. 1997; 1998), suggesting that the phenomenon is characteristic for prosimians. Although the cause for it is not clear, in *V. variegata* it has been associated with the lack of chorionic gonadotropin (CG, Shideler et al. 1983). Since the gene for CG appears to be restricted to anthropoid primates (Maston and Ruvolo 2002), it is most likely that all strepsirrhines (and tarsiers) do not secrete CG during pregnancy and that this is indeed responsible for the lack of early post-conceptual elevated estrogen levels observed in all prosimian species studied to date.

Estrogen excretion during the second half of pregnancy was strongly related to the sex of the offspring born, with only females that gave birth to a male infant showing elevated estrogen levels. A link between estrogen excretion and sex of the fetus in utero has also been described in the polytocous *V. variegata*, in which estrogen concentrations were strongly correlated with the number of male fetuses and associated with fetal testicular hyperplasia (Shideler et al. 1983). Indirect evidence for a similar phenomenon exists also for *E. mongoz* from the study of Curtis et al. (2000). Since *N. pygmaeus*, to our knowledge the only non-lemur species in which data on the relationship between maternal estrogen levels and fetal sex exist, does not show this phenomenon (Jurke et al. 1997), it might represent a unique reproductive characteristic of lemurs. More data on other lemur and non-lemur prosimians are, however, needed to confirm this assumption and to assess the phylogenetic origin of this trait and its evolutionary significance.

For the single-birth redfronted lemur, our data, however, provide clear evidence that estrogen measurements during the last six weeks of gestation allow a reliable prenatal sex determination. This has major implications for studies on the reproductive biology of the species. For example, it allows reliable assessment of the sex ratio at birth, which up to now is almost impossible to determine because

redfronted lemur infants, like infants of many other lemur species, are sexually monomorphic for the first 2 - 3 months of age (Ostner pers. obs.), a time by which, however, a high proportion of infants has already died (Ostner and Kappeler in prep.). The option of sexing infants prenatally, thus, opens the possibility to test hypotheses concerning sex ratio theory (e.g. Clutton-Brock and Iason 1986) and to collect data on sex-specific infant mortality in the first months of life.

In conclusion, the data presented in this study provide the first insights into the endocrinology of *E. fulvus*. By doing so, the study significantly extended our knowledge on the reproductive physiology of the species and, moreover, provided important data for comparison regarding lemur reproductive endocrinology in general. However, further investigations are required to fully characterize the endocrinological events during the breeding season and understand the behavioral and hormonal relationships associated with estrus in this interesting lemur species.

## ABSTRACT

In order to characterize the endocrine changes associated with ovarian function and pregnancy in wild redfronted lemurs (*Eulemur fulvus rufus*), profiles of immunoreactive 5 $\alpha$ -pregnane-3 $\alpha$ -ol-20-one (5-P-3OH) and total estrogens ( $E_t$ ) in feces were generated during the breeding season and gestation. HPLC data confirmed the presence of high amounts of 5-P-3OH in the feces of redfronted lemurs and showed that estrone and estradiol-17 $\beta$  were the major estrogens excreted into feces, with estrone predominating. Due to an extreme day-to-day variability in excretion of both hormones during the breeding season, estrus cycle characterization was not possible for wild females. Comparison with endocrine data from captive animals suggests that this may be associated with a highly variable amount of undigested fecal matter in the feces of wild females. A gestation length of 121.3 days was calculated on the basis of the day of behavioral estrus and date of parturition. Hormonally, pregnancy could be reliably diagnosed from day 40 - 45 after conception, when fecal progesterone levels clearly increased above breeding season concentrations in all females. Levels gradually increased thereafter, with 15 - 35 fold elevated maximum levels being reached shortly before parturition. In contrast, the pattern of fecal estrogen excretion was strongly related to the sex of the fetus in utero, with only females carrying male infants showing elevated (25 - 50 fold)

estrogen levels during the last third of gestation, while levels remained at low breeding season levels in female-carrying mothers. Thus, fecal estrogen measurements during the last 6 - 7 weeks of gestation allow fetal sex to be determined prenatally. By providing the first data on the reproductive endocrinology of *E. fulvus*, we have shown that fecal hormone analysis is a useful tool to monitor endocrine changes associated with pregnancy, while it appears to have certain limitations regarding the assessment of ovarian function.

## CHAPTER 2:

Seasonal variation and social correlates of androgen excretion  
in male redfronted lemurs (*Eulemur fulvus rufus*)

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## INTRODUCTION

Androgens, and in particular testosterone, influence a variety of morphological traits as well as physiological and behavioral processes in vertebrates. Testosterone is essential for the formation of secondary sexual characters, such as size or coloration of external genitalia (e.g. in *Crocuta crocuta*: Glickman et al. 1987, 1992; *Mandrillus sphinx*: Wickings and Dixson 1992), sex-dependent facial characteristics or plumage coloration (e.g. throat pouch and cheek pads in *Pongo pygmaeus*: Maggioncalda et al. 1999; coloration in *Agelaius phoeniceus*: Weatherhead et al. 1993; in *Passer domesticus*: Gonzales et al. 2001) or weapons, such as elongated canines or antlers in males (see Dixson 1998 for review). Furthermore, testosterone plays a crucial role in the initiation and maintenance of sperm production and sexual behavior (Wickings et al. 1986; Baum 1992) and has been linked to the expression of aggressive behavior and social status in birds and mammals (Harding 1981; Bouissou 1983; Albert et al. 1990). Thus, in the long run, variation in testosterone may influence the reproductive success of individual males by modulating their behavior and physiology.

In primates, the influence of seasonal as well as social factors on testosterone secretion has been investigated in a variety of species, although rarely in the wild. Many species show seasonal cycles of sexual activity, resulting in distinct mating and birth seasons, and males of most seasonal breeding primate species exhibit corresponding cycles of testicular endocrine activity. For example, elevated testosterone values during the mating season have been documented in a number of anthropoid primates (Wiebe et al. 1988; Lohiya et al. 1998; but see Strier et al. 1999) and in some prosimians (Perret 1992; Brockman et al. 1998; Kraus et al. 1999). Besides these seasonal effects on testosterone secretion, there also appears to be a close association of androgens with social factors, such as aggression or social status (Whitten 2000). The relationship between testosterone and aggressive behavior seems to be modulated by social factors. Under stable conditions, levels of testosterone often do not correlate with dominance status or aggressive behavior (Sapolsky 1982, Nieuwenhuijsen et al. 1987; Cavigelli and Pereira 2000), while such a correlation can be found in situations with unstable hierarchies or other social challenges (Sapolsky 1983; Steklis et al. 1986; Bernstein et al. 1992; Cavigelli and Pereira 2000; Brockman et al. 2001). This environmental modulation of the

interaction between androgens and aggression has been formulated in the “challenge hypothesis” (Wingfield et al. 1990; pp.833): “An increase in the frequency or intensity of reproductive aggression as an effect of T(estosterone) is strongest in situations of social instability, such as during the formation of dominance relationships, the establishment of territorial boundaries, or challenges by a conspecific male for a territory or access to mates.”

In addition to fluctuations in testosterone concentrations due to seasonal and social factors affecting all males, there may also be status-dependent differences in testosterone secretion between individual males. Such rank-related differences may serve as a mechanism of intrasexual reproductive competition, because androgens do not only influence the expression of sexual behavior but are also essential for sperm production and influence sperm quality (Wickings et al. 1986). By suppressing testicular endocrine activity of rival males, dominant males may increase their own reproductive success (e.g. Keverne et al. 1982; Arnold and Dittami 1997). Physiological suppression, facilitated via olfactory or stress-mediated mechanisms, has indeed been documented in *Microcebus murinus*, *Mandrillus sphinx*, *Propithecus verreauxi* and *Pongo pygmaeus*, where high-ranking males exhibit elevated levels of testosterone compared to low-ranking ones (Schilling et al. 1984; Izard 1990; Wickings and Dixson 1992, Brockman et al. 1998; Kraus et al. 1999; Maggioncalda et al. 1999). Dominance-related differences in androgen secretion were not found in a number of other primate species (e.g. *Cercopithecus aethiops*: Steklis et al. 1985; *Saimiri sciureus*: Steklis et al. 1986; *Macaca arctoides*: Nieuwenhuijsen et al. 1987) and although the reasons for these findings may be numerous, one reason may be that males of these species rely on other mechanisms of intrasexual competition.

Redfronted lemurs (*Eulemur fulvus rufus*) are an ideal study subject for the evaluation of androgen-behavior interactions in the wild. They are highly seasonal breeders with a distinct mating season limited to about 4-6 weeks in May and June during which each female is receptive for only a single day (Boskoff 1978; Overdorff 1998; Ostner and Kappeler 1999). Males provide no direct paternal care. Redfronted lemurs, like all gregarious Malagasy lemurs, deviate from most anthropoid primates by their lack of sexual dimorphism in body and canine size (Kappeler 1990; 1996), which indicates the importance of an indirect form of male-male competition instead of direct contest competition. In line with this argument are observations of relatively large testes in redfronted lemur males compared to other prosimians (Glander et al.

1992; Kappeler 1997). Furthermore, despite continuous spermatogenesis throughout the year, sperm production is clearly elevated during the mating period (Brun and Rumpler 1990; Rasamimanana et al. 1990). The combination of large testes size and lack of sexual dimorphism makes an indirect form of male mating competition, such as reproductive suppression or sperm competition, at least likely.

The social structure within redfronted lemur multi-male - multi-female groups has been described as pair-bonds between individual males and females in some captive groups (Kappeler 1993; Pereira and McGlynn 1997). In wild populations, however, either socially functional one-male groups were found with a dominant male monopolizing social and sexual interactions with females (Ostner and Kappeler 1999) or evidence of clear dominance relations among co-resident males was lacking altogether (Overdorff 1998; Gerson 2000). Despite the lack of sexual dimorphism in body and canine size, redfronted lemur males engage in aggressive interactions over access to receptive females during the mating season (Overdorff 1998; Ostner and Kappeler 1999). Data on reproductive success of wild redfronted lemur males revealed that the dominant male indeed fathered most of the offspring (Wimmer and Kappeler 2002). The existence of distinct social classes in at least one population and frequent aggressive interactions between co-resident males allows the first investigation of the social modulation of androgen excretion in a wild population of redfronted lemurs.

In this study we examine to what degree seasonal and social factors influence testosterone secretion and whether testicular endocrine suppression is involved in male-male reproductive competition. Non-invasive hormone measurements from fecal samples are an established method to investigate physiology-behavior interactions in wild primates (see Whitten et al. 1998 for review). In this respect, measurements of fecal testosterone have been shown to be particularly useful in providing information on male testicular endocrine function in captive and wild lemurs (*Propithecus verreauxi*: Brockman et al. 1998; Kraus et al. 1999; *Lemur catta*: Cavigelli and Pereira 2000; von Engelhardt et al. 2000), although they have not yet been applied to redfronted lemurs. We will show that clear dominance relations exist among co-resident males and that dominant males engage more frequently in aggressive, as well as sexual behavior, both of which have previously been shown to be associated with variations in testosterone levels. Based on this information, we test the following predictions: (1) As a correlate of indirect male intrasexual

competition immunoreactive testosterone (iT) levels will be higher in dominant than in subordinate males. (2) In seasonally breeding redfronted lemurs iT levels will be higher during the mating than during the pre-mating season. (3) Overall iT values will be positively correlated with rate of male-male conflicts. (4) According to the “challenge hypothesis”, iT levels will be elevated during unstable situations and will be paralleled by an increase in male aggression. (5) Elevated iT levels will be correlated with the rate of group encounters as these may constitute challenging situations for resident males.

## METHODS

### Study site and population

The study was conducted in Kirindy Forest, a dry deciduous forest located in Western Madagascar within a forestry concession operated by the Centre Formation Professionnelle Forestière de Morondava. The Kirindy area is characterized by pronounced seasonal changes with a hot rainy season from November to March/April and a cooler dry season between May and October (for a detailed description of the forest see Ganzhorn and Sorg 1996). Within a 50ha study area all animals from 5 groups are habituated to human presence and individually marked with nylon or radio collars. All groups have been subjected to regular censuses since 1996 (Kappeler, unpubl. data). Animals with unknown birth dates were aged according to their body weight and dental wear (see Richard et al. 1991). All animals older than 32 months were considered adult (Sussman 1977). This study covers the period from May 1999 to June 2000 including two mating seasons and focuses on three groups living in adjacent home-ranges.

Table 1: Composition of study groups, fecal samples collected, observation hours and phases of social instability

group	adult males	adult females	juveniles or infants	number of fecal samples	observation hours	social instability
A	4-5	2-3	3-5	419	1065	Jun 00
B	2-4	2-3	3-5	385	1053	Feb/Mar 00
F	3	2	3	81	520	Jun 00

## Focal groups

The composition of the three study groups (A,B,F) over the course of the study period is depicted in Table 1. In group A, one male died at the end of August 1999 and three males emigrated from the group during the mating season in June 2000. In group B, two males emigrated and one male immigrated in February 2000.

## Behavioral observations

Behavioral data were collected on groups A & B over the entire study period, while observation of group F started in October 1999. Agonistic interactions and sexual behavior (copulations) were recorded via one-hour focal animal sampling (Altmann 1974), yielding a total of 2638 hours of focal data. Observations were equally distributed among all adult group members and all hours of the day (dawn to dusk). In general, the observation routine rotated among the three study groups with each group contributing focal animals for an entire day before continuing with another group the following day. Observations were made throughout the study period, but observation frequency increased during the mating season as the most important time for the aims of this study. During the mating season 1999 observations were made on two groups simultaneously with the help of an assistant. Distribution of behavioral observations across the different groups and months of the study are shown in Table 2. Interactions were defined as agonistic, if at least one opponent showed aggressive (lunge, charge, cuff, grab, bite, chase) or submissive (be displaced, cower, jump away, flee) behavior (*sensu* Pereira and Kappeler 1997).

Table 2: Observation time and fecal sample collection distributed across the three study groups and across the months of the study

	observation hours			fecal samples		
	A	B	F	A	B	F
May 99	132	132		41	47	
Jun 99	208	205		59	46	
Jul 99	72	72		38	26	
Aug 99	34	39		34	24	
Sep 99	50	50		23	24	
Oct 99	60	70	36	25	28	
Nov 99	56	67	64	24	23	
Dec 99	74	67	50	24	23	
Jan 00	60	59	50	24	23	
Feb 00	60	65	50	24	15	
Mar 00	4	9	34	16	18	
Apr 00	56	53	16	18	20	
May 00	99	76	110	45	30	35
Jun 00	100	89	110	24	38	37
total	1065	1053	520	419	385	72

Data on group encounters were collected throughout the study period. Specifically it was recorded whether the encounter led to inter-group fighting and if so, which animals were involved. Additionally, the duration of the encounter and the area, in which the conflict took place, was recorded as well as which group eventually won the conflict, i.e. drove the other group away.

### **Phases of social instability**

Stable and unstable situations could be easily distinguished from each other: While during phases of social stability the ranks of individuals were stable and the directional consistency of the outcome of agonistic interactions was very high, unstable situations were characterized by a reorganization of the hierarchy. In three of the four study groups a phase of social instability occurred within the study period. Two of these periods of instability followed emigration or immigration of males. After the emigration of three males from group A at the end of the study in June 2000 only one male stayed in the group. Immediately after the emigration, an unmarked male tried to evict the remaining single resident male. Aggressive interactions lasted several days and data collection ended while the situation was still unclear. In group B following the emigration of two males the immigration of a single male was observed in February 2000. Approximately 2 months later the immigrated male took over the top-ranking position. In group F a period of social instability and rank reversals among the resident males occurred at the end of the mating season in June 2000.

### **Collection of fecal samples and immunoreactive testosterone analysis**

Fecal samples were collected from all 13 adult males of the study groups. In groups A and B samples were collected over the entire study period from May 1999 to June 2000, while samples of group F were only collected during the mating season 2000 (Tab. 2). Frequencies of sample collection varied according to season from every third day (mating season May-June) to every fifth day (non-mating season), yielding a total of 884 samples (27 - 119 samples per male). Samples were collected immediately after defecation and preserved in 8 ml absolute ethanol until hormone analysis (see Kraus et al. 1999).

Prior to hormone measurements samples were mechanically homogenized in their original ethanolic solvent and subsequently extracted twice by shaking overnight as described by Ziegler et al. (2000a). Efficiency of the extraction procedure, determined in a subset of 30 samples by monitoring the recovery of  $^3\text{H}$ -progesterone (40,000 cpm) added to the samples prior to homogenization, was  $75.7 \pm 6.80\%$ . Although recovery figures might be slightly underestimated by the use of progesterone rather than radioactive testosterone, its use was preferred given the potential risk of co-measurement of externally added testosterone tracer in the highly sensitive testosterone assay used (see below). Fecal extracts were measured for immunoreactive testosterone by microtiterplate enzymeimmunoassay (EIA) using an antiserum raised against testosterone-3-(O-carboxymethyl)oxime-BSA (purchased from BioClinical Services Ltd, Cardiff, UK) and biotinylated testosterone as a label. The antibody was chosen as it has been successfully applied to fecal androgen measurements in the related ringtailed lemur (von Engelhardt et al. 2000), suggesting its suitability in other lemurs. Moreover, an initial comparison of the levels of fecal androgen immunoreactivity measures in samples from juvenile and adult males using this antibody showed that levels in the latter were significantly higher, indicating that the fecal androgen measurement used is of biological significance in reflecting testicular function also in redfronted lemurs.

The assay procedure has been described by Kraus et al. (1999). In brief, fecal extracts were diluted 1:200 in assay buffer (0.04 M PBS, pH 7.2) and 50 $\mu\text{l}$  aliquots taken to assay along with 50  $\mu\text{l}$  aliquots of reference standard in doubling dilutions in the range of 0.15 – 20 pg/well. Serial dilutions of fecal extracts from samples of different males gave displacement curves parallel to that obtained for the testosterone standard. Sensitivity of the assay at 90% binding was 0.5 pg and intra- and inter-assay coefficients of variation of high and low value quality controls were 7.2% (n = 16) and 11.0% (n = 62) (high) and 10.3% (n = 16) and 14.0% (n = 60) (low), respectively.

### **Data analyses**

The mating season was defined as the period between the first week of May and the last week of June. 89% of observed copulations (n = 222) occurred within this period and dates of estrus for each female (calculated by counting backwards

from dates of subsequent births) also fell within this time interval. The time period 4 months prior to the mating season was defined as pre-mating season.

Dominance relations among males were determined on the basis of decided agonistic interactions. Agonistic interactions were considered decided if one opponent showed only submissive behavior, while the other did not (Hausfater 1975; for *E. fulvus* see Pereira et al. 1990). According to the outcome of these interactions a dominance matrix was established for each study group. The degree of directional consistency was calculated - whenever group size allowed testing - using a test implemented in Matman (de Vries et al. 1993; de Vries 1995). For a comparison of androgen excretion with rate of male-male aggression over the course of the study, the group mean of male-male conflicts was generated as the average of the individual mean aggression rate per month for each male. Rates of group encounters were calculated for each group using the frequency of encounters per month in relation to the time these groups were observed.

Data were analyzed using parametric statistical tests, although in some cases the data did not conform to all assumptions for the use of such tests. However, the statistical tests used here are considered very robust against violations of the assumptions and therefore their use seems justified (Sokal and Rohlf 1981). Rank-related differences in aggressive and sexual behavior, as well as androgen levels were tested using t-tests. The effect of season on androgen excretion was analyzed with an ANOVA for repeated measures. The relations between rate of aggression and androgen excretion as well as rate of group encounter and androgen excretion were investigated with Pearson Product-Moment correlation tests. All tests are implemented in Statistica 6.0<sup>®</sup> and were performed two-tailed. Descriptive statistics are reported as means and standard deviation (SD).

### **Sample size**

The respective sample sizes for dominant and subordinate males vary according to the time period and parameter tested. For example, in the three study groups there were altogether four dominant males, because in one group (B) the dominant position was taken over by an immigrating male and the former dominant male stayed as subordinate in the group. As a consequence, this male entered the analysis in one mating season as dominant and in the next as subordinate. We assume the data points to be independent, because the variable under investigation



was not the individual but the animal's dominance position, which is a relational and not a fixed quality. Sample size in general was larger if behavioral parameters were tested than if androgen values were needed for analysis, because more males were subject to behavioral observation than to fecal sample collection.

## RESULTS

### Dominance relations among males

There were clear dominance relations among co-resident males. The percentage of decided conflicts was very high, especially within male-male dyads (mean of three groups:  $92 \pm 4$ ). The directional consistency of the outcome of male-male conflicts reached the highest possible value (1.0) over the entire study period, except during phases of social instability, meaning that all dominance positions were clearly defined and there were no deviations from the winner-loser pattern for any dyad.

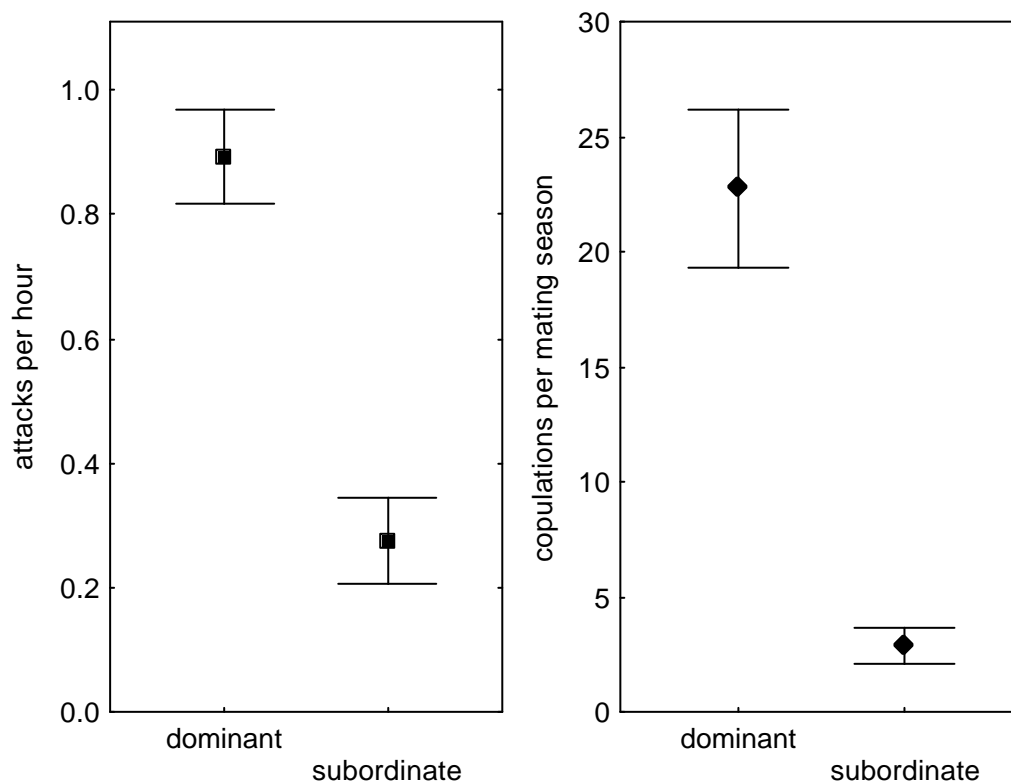


Figure 1a: Male rank and social behavior: Mean ( $\pm$  SD) hourly rate of attacks of dominant ( $n = 4$ ) and subordinate ( $n = 9$ ) males throughout the study period. Only focal observations entered the analysis. 1b: Mean ( $\pm$  SD) frequency of copulations of dominant ( $n = 4$ ) and subordinate ( $n = 9$ ) males. Only focal observations entered the analysis.

The seasonal pattern of fecal iT excretion followed a similar course for all males. We found a significant effect of season on iT with peak levels being observed in both mating seasons and around the birth season in September/October (repeated measures ANOVA:  $F_{13, 65} = 16.94$ ,  $p < 0.01$ ; Fig. 2). There was a significant difference in fecal iT levels of males between the pre-mating season ( $69.22 \pm 15.39$ ,  $n = 9$ ) and the mating season ( $186.13 \pm 49.17$ ,  $n = 15$ ; t-test:  $t = 6.88$ ,  $p < 0.01$ ; Fig. 3). Interestingly, the concentrations of fecal iT in the birth season ( $262.75 \pm 44.29$ ,  $n = 8$ ) were significantly higher than the concentration during the mating seasons (t-test:  $t = -3.68$ ,  $p < 0.01$ ).

In each group we could identify a dominant male, who was winner in all decided conflicts, all other males were defined as subordinates. We did not assign further rank positions among subordinates. Dominant males initiated significantly more attacks per hour ( $0.89 \pm 0.15$ ,  $n = 4$ ) than did subordinate males ( $0.27 \pm 0.21$ ,  $n = 9$ ; t-test:  $t = 5.27$ ,  $p < 0.01$ ; Fig. 1a), indicating that dominant males not only won all conflicts they were involved in, but also behaved more frequently in an aggressive way than subordinate males did. In addition, dominant males engaged significantly more often in sexual behavior ( $22.75 \pm 6.95$ ,  $n = 4$ ) than subordinate males ( $2.89 \pm 2.37$ ,  $n = 9$ ; t-test:  $t = 7.96$ ,  $p < 0.01$ , Fig. 1b).

### **Social status and immunoreactive testosterone levels**

In contrast to our prediction we did not detect any differences in fecal iT concentrations between the two classes of males during the mating season (dominant:  $n = 4$ ,  $185.00 \pm 46.51$ ; subordinate:  $n = 8$ ,  $176.88 \pm 56.09$ ; t-test:  $t = 0.25$ ,  $p = 0.809$ ). Hormone data for the males of group F were omitted in this analysis because rank reversals in the second half of the mating season made classification by rank for these males impossible. Nevertheless, all three males of group F exhibited similar iT levels ( $212.33 \pm 36.02$ ,  $n=3$ ) and their values did not deviate significantly from those of males from other groups ( $179.58 \pm 51.07$ ,  $n = 12$ ; t-test:  $t = -1.03$ ,  $p = 0.320$ ).

### Seasonal variation in immunoreactive testosterone levels

The seasonal pattern of fecal iT excretion followed a similar course for all males. We found a significant effect of season on iT with peak levels being observed in both mating seasons and around the birth season in September/October (repeated measures ANOVA:  $F_{13, 65} = 16.94$ ,  $p < 0.01$ ; Fig. 2). There was a significant difference in fecal iT levels of males between the pre-mating season ( $69.22 \pm 15.39$ ,  $n = 9$ ) and the mating season ( $186.13 \pm 49.17$ ,  $n = 15$ ; t-test:  $t = 6.88$ ,  $p < 0.01$ ; Fig. 3). Interestingly, the concentrations of fecal iT in the birth season ( $262.75 \pm 44.29$ ,  $n = 8$ ) were significantly higher than the concentration during the mating seasons (t-test:  $t = -3.68$ ,  $p < 0.01$ ).

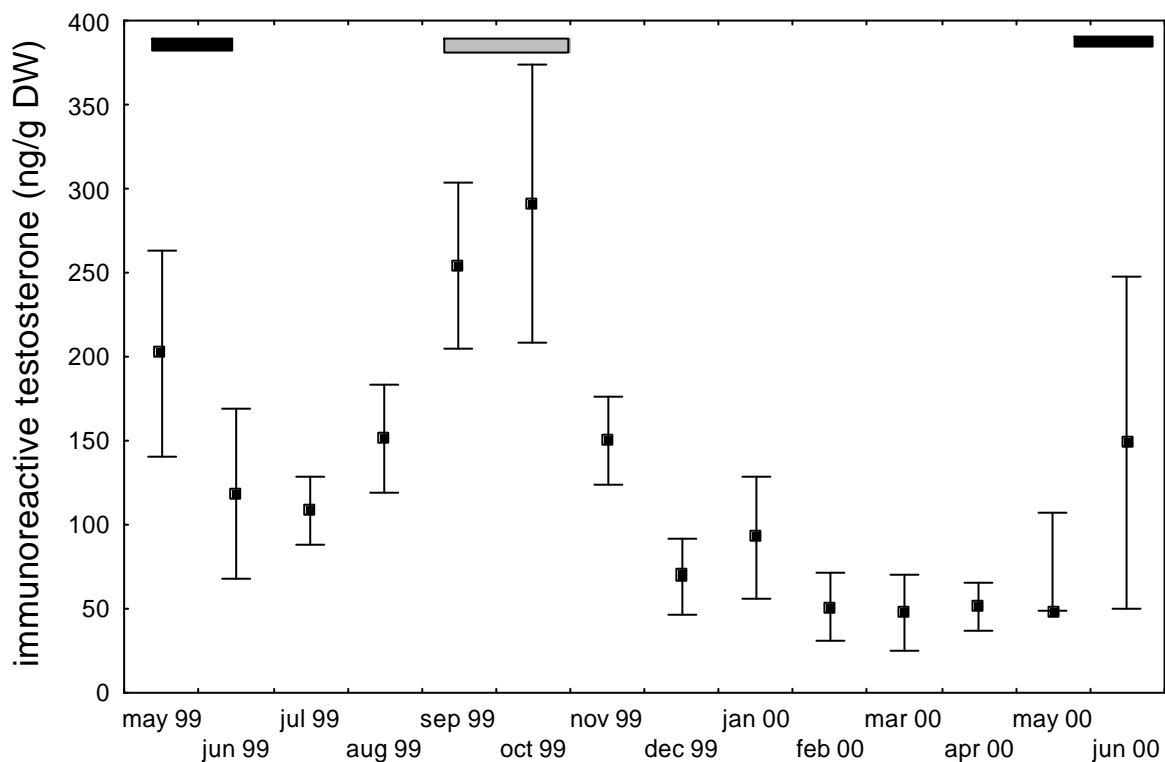


Figure 2: Seasonal variation in mean ( $\pm$  SD) fecal immunoreactive testosterone concentrations of 6 males over 14 months. Only males who stayed in the same groups over the entire period and could hence be sampled regularly were considered here. Black bars indicate mating seasons, the hatched bar the birth season.

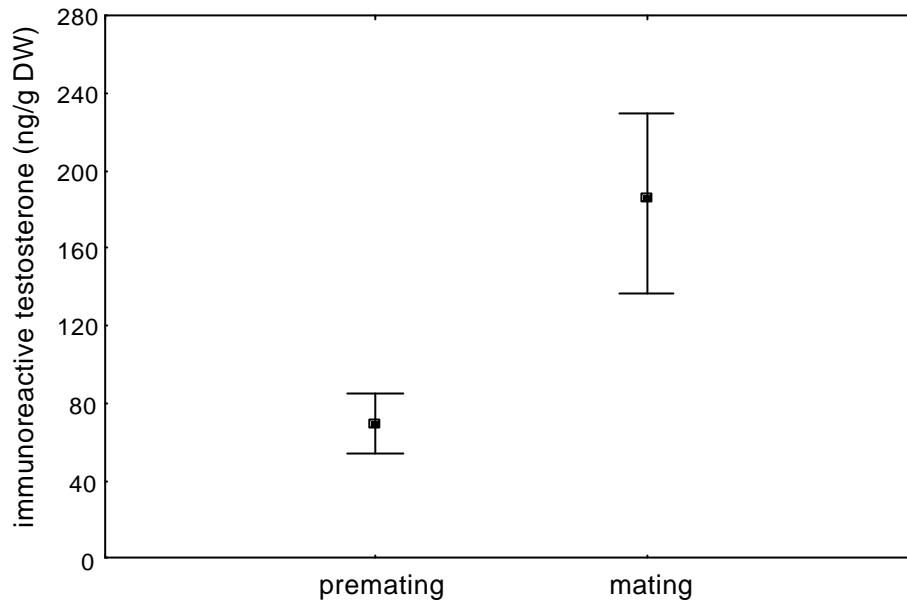


Figure 3: Differences in fecal immunoreactive testosterone concentration ( $\pm$  SD) between the pre-mating ( $n = 9$  males) and the mating season ( $n = 15$  males). Samples of all males (dominant and subordinate) entered the analysis and data of both mating seasons were pooled.

### Rate of aggression and immunoreactive testosterone

The temporal pattern of the rate of male-male conflicts did not parallel the monthly iT excretion. There was no significant correlation between the two parameters (group A:  $n = 14$ ,  $r = 0.22$ ,  $p = 0.460$ ; group B:  $n = 14$ ,  $r = -0.29$ ,  $p = 0.315$ ). While in all groups iT concentrations were highest during the mating and the birth season, rates of male-male conflicts reached their maxima in group A and F in June 2000 (mating season) and in group B in February and March 2000 (pre-mating season; Fig. 4). These phases were in all three cases the periods of social instability. While the increase in male-male conflicts in groups A and F during these unstable periods within the mating season paralleled the overall increase in androgen concentrations, this was not the case in March 2000 in group B, well outside the mating period.

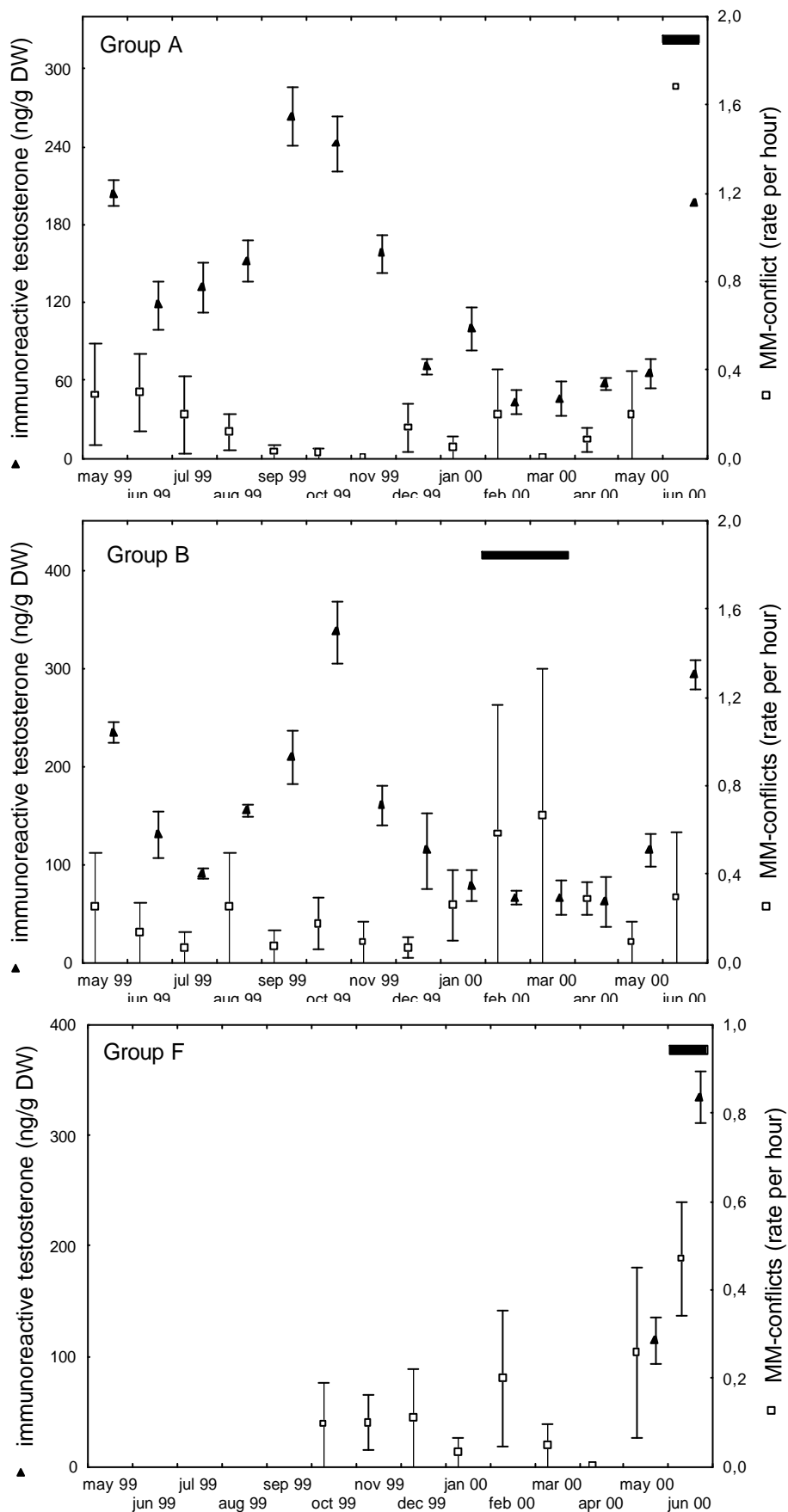


Figure 4: Temporal pattern of rates of male-male conflicts and fecal immunoreactive testosterone concentrations in three study groups. Depicted are monthly means with standard error (SEM). Phases of instability are marked with black bars. Note that in group F data were not collected over the entire study period.

After the emigration of two subordinate males from group B at the end of January 2000 an adult male (THA) immigrated into this group on February, 13<sup>th</sup>. Although male THA took the gamma position in the male dominance hierarchy (below dominant BAL and subordinate HON) and dominance relations were clear-

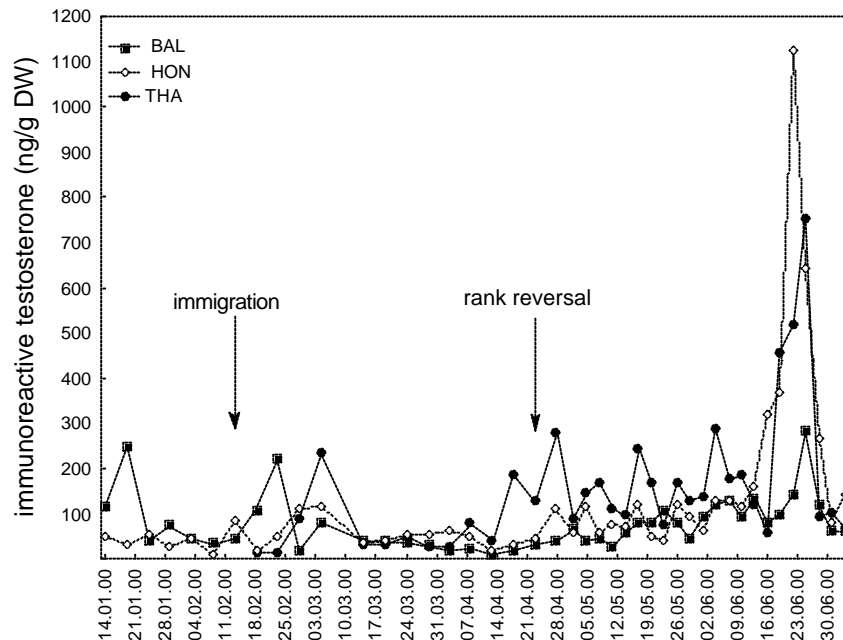


Figure 5: Individual profiles of immunoreactive testosterone of three males of group B during the period of social instability following the immigration of male THA and subsequent rank reversal between former dominant male BAL and subordinate male THA (see text for details).

cut, the rate of male-male conflicts increased substantially, while testosterone levels remained unchanged for several weeks (Fig. 5). Approximately two months after THA's immigration the dominant male BAL was observed one morning with severe injuries and THA had taken over the top-ranking position. This rank reversal was accompanied by a phase of slightly elevated androgen levels of male THA while the excretion of the other two males remained unchanged. However, comparable short-term elevations as seen during this phase can be observed also before and after the rank reversal for male THA as well as for BAL and, therefore, can not be linked clearly to this event. Moreover, if males reacted to challenging situations with increases in androgen excretion we would also expect to see an elevation in the androgen levels of the challenged male BAL, which was not the case. For a more detailed analysis of the relationship between aggression and androgen concentration, we compared the frequency of overall aggression (attacks against any

group member) of each male with the corresponding iT level during each mating season, as this is the time of most pronounced sexual and aggressive behavior. As indicated in Figure 6, there was no significant correlation between these variables ( $n = 15$ ,  $r = 0.17$ ,  $p = 0.551$ ).

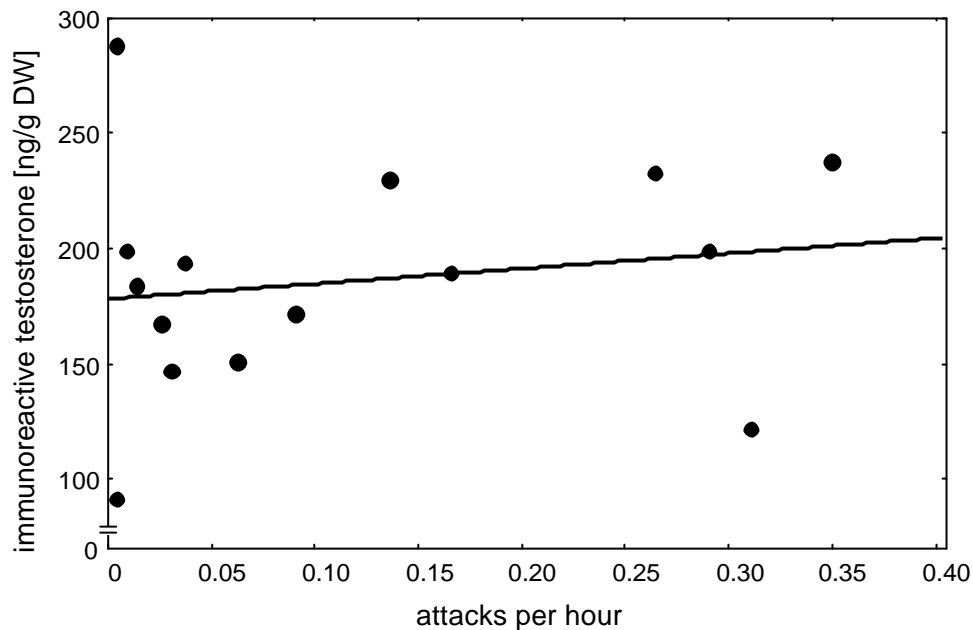


Figure 6: Individual male immunoreactive testosterone concentrations relative to corresponding rates of initiated attacks during the two mating seasons ( $n = 15$ ). Each data point depicts the mean value of iT level and attack rate for one male and during one mating season, but data from both seasons entered the analysis.

Because we assumed that group encounters may constitute challenging situations for resident males we compared the monthly rate of group encounters with the monthly mean iT values of all males of this group. The rate of encounter did not correlate significantly with fecal iT excretion ( $n = 29$ ,  $r = -0.22$ ,  $p = 0.251$ ). Dominant males face the highest possible costs, i.e. loss of their rank position, group or potential offspring, and thus we compared the individual androgen levels of dominant males with group encounter rates; again, no significant correlation could be detected ( $n = 27$ ,  $r = -0.06$ ,  $p = 0.770$ ).

## DISCUSSION

### Dominance relations among males

Clear dominance relations existed among males with a dominant male in each of the three study groups, who was winner in all conflicts. Two males who were

dominant at the beginning of our study remained in this position, while the remaining two were replaced by an immigrant male in one case and a former subordinate male in the other case. These data clearly show for the Kirindy population of redfronted lemurs that males fall into two distinct social classes, a finding which is in accordance with that of our previous study on the same population based on more limited data (Ostner and Kappeler 1999). These results are, however, in contrast to the findings of other studies from both captivity and the wild, in which clear dominance relations among males were not found (Kappeler 1993; Pereira and McGlynn 1997; Overdorff 1998) and emphasize the remarkable flexibility in social structure exhibited by redfronted lemurs (see also Pereira and Kappeler 1997).

In the present study dominant males were involved more frequently in agonistic and sexual interactions than subordinate males, thus confirming previous findings on the same population (Ostner and Kappeler 1999). These data, however, deviate from observations of exclusive pair-bonds and mating privileges among pair-partners in captive redfronted lemurs (Kappeler 1993; Pereira and McGlynn 1997). Hence, male redfronted lemurs in Kirindy competed directly for access to receptive females and dominant males enjoyed mating privileges. However, dominant males did not monopolize access to females exclusively for themselves, because most subordinate males were also observed copulating, although significantly less frequently. A positive correlation between dominance rank and male mating success has been found in a number of primate species (Cowlshaw and Dunbar 1991). Whether mating success translates directly into reproductive success can only be resolved by genetic analyses. Paternity analyses on the same population of redfronted lemurs indicate that dominant males fathered most of the offspring (Wimmer and Kappeler 2002).

### **Effects of social status on androgen excretion**

In contrast to our expectation, social status was not linked with levels of iT as dominant and subordinate males exhibited similar iT values throughout the entire reproductive cycle, including the mating season. This result deviates from the findings of a number of studies on different vertebrate taxa (reviewed in Holekamp and Smale 1998), including both anthropoid and prosimian primates (e.g. *Mandrillus sphinx*: Wickings and Dixson 1992; *Propithecus verreauxi*: Kraus et al. 1999; *Lemur catta*: Cavigelli and Pereira 2000), in which clear rank-related differences in androgen



levels among males were observed. However, there are also a number of studies on other species in which no relationship between social status and testosterone was found, although social positions were clearly defined (*Crocota crocota*: Holekamp and Smale 1998; Goymann et al. 2001; *Saimiri sciureus*: Lyons et al. 1994; *Gorilla gorilla*: Robbins and Czekala 1997; *Macaca fascicularis*: van Schaik et al. 1991; Morgan et al. 2000).

In contrast to other lemur species, such as *Propithecus verreauxi* (Brockman et al. 1998; Kraus et al. 1999) and *Microcebus murinus* (Schilling et al. 1984), dominance-related suppression of testicular endocrinology is unlikely to be involved in intrasexual competition between males in redfronted lemurs. However, redfronted lemur males may apply other tactics. One indicator for a different indirect mechanism of male-male competition is the relatively large size of redfronted lemurs' testes (Glander et al. 1992; Kappeler 1997). It seems possible that the two gregarious lemur species for which these sets of data are available – *P. verreauxi* and *E. fulvus rufus* – apply two different mechanisms of intrasexual competition – the former using physiological suppression expressed by differences in testosterone levels linked with relatively small testes size and infrequent matings (Brockman et al. 1998; Kraus et al. 1999), whereas the later competes via sperm competition, as suggested by large testes and frequent matings.

The lack of a status-dependent difference in iT levels may also be due to the low power of the test performed as it was based on a small sample size (4 dominants and 8 subordinates). With an alpha of 0.05 and a medium effect size (= 0.50; see Cohen 1988) the power of the test was 0.11 (calculated using G\*Power; Buchner et al. 1997). Although the difference between the means of the two respective social classes was in the predicted direction, it is small, while the standard deviation is high. This leads to an extreme overlap of samples making it unlikely that the prediction was unsupported due to low statistical power. The high p-value (0.81) is further evidence for the correctness of our result. If endocrine differences between dominants and subordinates were linked to differences found in aggressiveness and sexual behavior we would expect them to be much more clear-cut and the difference in iT level to be substantial, both of which are not the case in our sample.

As the problem of small sample size applies to some of the other predictions, we also calculated statistical power analyses for other tests. Four power analyses revealed statistical power of 0.19-0.37 depending on the respective sample size (min

= 14; max = 29) and a medium effect (= 0.30). Monthly rate of aggression and iT levels were not significantly correlated in either group. In the case of group A the results were in the predicted direction. We cannot exclude that the non-significant result is due to low statistical power. In group B, however, the direction of the relationship runs counter to the prediction and we would expect the general prediction to hold for both groups under the same conditions. Rate of aggression and iT levels during the mating season are also related in the predicted direction, but the p-value is relatively high. In the last case of a non-significant result (rate of group encounter and iT levels) the direction of the relationship is counter to predictions, confirming our finding of no relationship between the variables.

### **Seasonality of androgen excretion**

The seasonally restricted reproduction of redfronted lemurs (van Horn and Eaton 1979) was mirrored by a seasonal pattern of androgen excretion. During the mating season, males exhibited a 2 - 3 fold increase in fecal iT compared to the pre-mating period - a pattern also found in other seasonally breeding lemur species (Perret 1992; Brockman et al. 1998; Kraus et al. 1999). These data are in line with the observation of increased sperm production during the mating season in *E. fulvus* (Brun and Rumpler 1990), which is also mirrored by an observable increase in testis size during the period of sexual activity (J. Ostner, pers. observation). Therefore, our data indicate that testosterone is linked to spermatogenesis in redfronted lemurs, as has already been confirmed for other primate species (Wickings et al. 1986)

Significant increases in androgen concentrations were not exclusively correlated with the onset of the mating season, but did also occur during the birth season. Birth season iT values even exceeded those recorded in the mating season by 37%. Elevated levels of testosterone during the birth season have also been found in *P. verreauxi* as a consequence of dispersal events (Brockman et al. 2001), indicating that testosterone excretion may also be triggered by social, non-reproductive factors. The birth season may be an extremely stressful time period for males, if they have to protect infants against non-resident, potentially infanticidal males. The possibility of infanticide may promote elevated androgen levels in males as a means of supporting aggressive behavior against infanticidal males and also in their potential act of attacking infants in other groups themselves. Infanticide has been observed in several lemur species, including *E. fulvus rufus* (Pereira and Weiss

1991; Erhart and Overdorff 1998; Jolly et al. 2000). Although infanticide has not yet been directly witnessed in the Kirindy population, there is strong indirect evidence for its occurrence because on several occasions infants disappeared directly after new males immigrated into a group.

The finding of elevated levels of androgen during the birth season seems to be in contrast to predictions made by the “challenge hypothesis”, in which low levels of testosterone are expected as a trade-off between aggression and paternal care (Wingfield et al. 1990). While this seems to apply to almost all avian species (e.g. Hegner and Wingfield 1987; Vleck and Brown 1999; but see Peters et al. 2001), the evidence is mixed for mammals: Decreased levels of testosterone increased the rate of paternal behavior in some rodents (Brown et al. 1995; Clark and Galef 1999; Reburn and Wynne-Edwards 1999), whereas in other studies paternal behavior decreased following castration (Wang and de Vries 1993; Trainor and Marler 2001) or no effect was found (Lonstein and de Vries 1999). Two studies on primates with high levels of paternal care also yielded inconsistent results: While in *Callithrix kuhlii* the frequency of infant carrying and testosterone concentration was negatively correlated (Nunes et al. 2001), male *Saguinus oedipus* exhibited an increase in testosterone levels during the gestation period, which stayed elevated after parturition (Ziegler and Snowdon 2000).

Behavioral or physiological differences of birds and mammals may account for differences in the applicability of trade-off models. Many mammalian species with direct paternal care exhibit a post-partum estrus (see van Noordwijk and van Schaik 2000 for review) and males have to be fertile shortly after parturition (Ziegler et al. 2000b). In this case selection should favor aggression with possible elevations in testosterone levels if males have to defend fertile females against other males. In the same sense should the risk of infanticide and the need to remain aggressive in order to protect the infants also select against a trade-off between aggression and paternal care, even in the absence of a post-partum estrus. Gregarious lemurs like *P. verreauxi* and *E. fulvus rufus* exhibit no direct paternal care and no post-partum estrus but infants face a risk of infanticide. Therefore, we conclude that for redfronted lemurs in a situation with a potentially high infanticide risk due to the presence of newborn infants and the lack of direct paternal care the findings of high androgen values during the birth season are not unexpected.

### **Social instability, rate of aggression and androgen excretion**

The frequency of male-male conflicts peaked as predicted by the “challenge hypothesis” during phases of social instability, either following the immigration of a new male (group A and B) or due to rank reversals among resident males (group F). Two of the phases of social instability occurred during the mating season (group A and F) and were accompanied – as predicted – by an increase in iT excretion. However, increased levels of iT and aggression during the mating season could be independent from each other if the rise in androgen is only associated with the onset of testis growth and reproductive activity. During the phase of intense male-male fighting outside the mating season, which eventually led to a rank reversal in group B, neither the time of immigration nor the period around the rank reversal two months later was paralleled by extraordinary elevations in androgen excretion by any male. It is possible that the immigration of a male into a group may not impose a threat for the resident, dominant male, if the immigrating male enters low in the hierarchy, as it was the case in this study. Following immigration dominance relations were stable with frequent male-male conflicts initiated and won by the dominant male. The period of rank reversal, however, was clearly a challenging situation and it is unclear why the dominant male did not respond with elevated androgen excretions to this situation.

Because levels of iT and rate of within-group male-male conflict were not correlated, we assume that the real challenge for male redfronted lemurs may be imposed by extra-group males. Therefore, we expected the rate of group encounters to be predictive of the level of iT, especially during the birth season when non-resident males may pose an increased risk of infanticide. Our data, however, do not support this prediction, as we could not find a positive correlation between iT levels and frequency of group encounters. However, redfronted lemur territories highly overlap (Ostner and Kappeler in prep.) and groups meet frequently all year round (on average about once a day; 0.08 encounters/hour). Changes in encounter rate may therefore not impose an extra-challenge to males, because encounter rates are always relatively high. Moreover, these encounters usually occur between familiar neighbors and seem to pose no great risks, because aggressive interactions between members of neighboring groups occurred only in about 20% of cases (Ostner, unpubl. data). Hence, we conclude that group encounters constitute no

particularly challenging situation associated with an increased potential risk of group take-over or infanticide.

It thus appears, that while androgens may facilitate seasonal reproductive aggression, its occurrence outside the mating season (group B) does not necessarily require increased androgenic activity, which is in contrast to findings of simultaneously increased levels of aggression and testosterone in *Propithecus verreauxi* during the birth season (Brockman et al. 2001). Studies on birds, however, support our results, because there non-reproductive aggression also occurred despite low levels of testosterone (Burger and Millar 1980; Schwabl and Kriner 1991). It may be that group encounters and rank reversals do not constitute predictable situations of social challenges, especially if the rule for male immigration is to remain low in the hierarchy instead of taking over the alpha position, as it is the case in other primate species (e.g. *Semnopithecus entellus*, Borries 2000). At least in the Kirindy population the mode of male migration seems to reflect this pattern, because in all cases of immigration of a single male, the male entered the hierarchy at the bottom position and did not necessarily attempt to move up in rank immediately (unpubl. data). The relatively rapid rank reversal observed in this study in group B may have constituted an unpredictable situation to which the males did not react with changes in androgen excretion. It seems possible that during non-reproductive phases the coupling of aggressive behavior and endocrine production is hindered by a general attenuation of gonadal function outside the reproductive periods which prevents androgens to drive or respond to short-term challenges.

As expected, redfronted lemur males exhibited higher levels of iT during the mating season. However, they did not show status-dependent differences between dominant and subordinate males ruling out the possibility of dominance-related suppression of testicular endocrine activity as a means of male sexual competition. Androgen levels were not correlated with the overall rate of aggression and were only elevated if the period of social instability coincided with the mating season, as predicted by the “challenge hypothesis”. During other periods of social instability aggression rates increased while iT levels stayed the same. It seems possible that redfronted lemur males do not react with increases in androgen to short-term challenges, such as rank reversals or group encounters, and rises in androgen are instead correlated with longer-lasting and predictable situations, such as the mating

or birth season. To understand the impact of short-term challenges, it seems necessary to investigate the influence of other stress- or challenge-related endocrine profiles.

## ABSTRACT

The aim of this study was to examine effects of seasonal and social factors on male androgen excretion in a seasonally breeding primate living in multimale-multifemale groups. By combining detailed behavioral observations (>2500h) on three groups of redfronted lemurs living in Kirindy Forest/Madagascar with non-invasive hormone analysis of >800 fecal samples collected concomitantly of the same animals, we tested predictions on (1) the effect of social status on immunoreactive testosterone (iT) excretion, (2) seasonal variation of iT across reproductive periods and (3) the relationship between aggression and iT excretion. The study lasted 14 months, covering two mating and one birth seasons. The results revealed that males fall into two distinct social classes, with one dominant male and several subordinate males in each group. In contrast to our prediction, the behavioral differences between these two classes were not reflected by differences in androgen levels, making physiological suppression of testicular function an unlikely mechanism of male reproductive competition. As expected for a seasonally breeding animal, iT values were elevated during the mating season. Androgen levels tracked the increase in the rate of reproductive aggression during the mating season as predicted by the “challenge hypothesis”. An increase in aggression due to spontaneous social instability outside the mating season, however, was not linked to a parallel rise of iT. Furthermore, the highest iT levels were obtained during the birth season, which may be part of a male strategy to remain aggressive during this period of high infanticide risk. These findings suggest that redfronted lemurs do not respond with increases in androgens to short-term challenges and that high androgen levels instead correlate with longer-lasting and predictable situations, such as the mating and birth season.

## CHAPTER 3:

### SOCIAL THERMOREGULATION IN REDFRONTED LEMURS (*EULEMUR FULVUS RUFUS*)

**JULIA OSTNER**

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## INTRODUCTION

The climate of Madagascar is characterized by pronounced seasonality with temperatures dropping as low as 5°C during the dry season (Sorg and Rohner 1996; Wright 1999). Cold nights and the combination of low basal metabolic rate and high body temperature (Daniels 1984; Müller 1985) led to the hypothesis that lemurs respond to cold stress by inactivity and social thermoregulation, i.e. resting in tight body contact with one or several conspecifics, in order to conserve energy (Morland 1993; Pereira et al. 1999). A correlation between seasonal variation in activity pattern and ambient temperature has been documented for a variety of lemur species, including redfronted lemurs (Tattersall 1976; Morland 1993; Donati et al. 1999; Pereira et al. 1999). Pereira et al. (1999) further illustrated the need to conserve energy during the cold months with low food availability by their study on *Lemur catta*; ringtailed lemurs grow subcutaneous fat deposits and accelerate hair growth during the energy rich months.

Lemurs groups are smaller than those of most anthropoid primates of the same body size and as a result are comprised of only a few adults (Kappeler and Heymann 1996). Within multimale-multifemale groups the adult sex ratio usually is even or slightly male-biased (Kappeler 2000c). Such a ratio is unexpected considering male competition for access to receptive females. Also from the females' perspective surplus males may constitute a burden due to increased within-group feeding competition. It has been a longstanding issue in socioecology whether group composition is determined by males, females or both sexes and the discussion continues (Kappeler 2000b). In redfronted lemurs this issue has not been resolved yet, but in the case that females can regulate group composition, e.g. by the degree of estrus synchrony within a group, it can be expected that males provide females with a service, otherwise females should chose to live with only one male in order to decrease feeding competition. Females may in some cases also benefit from extra females in their groups, but they should generally prefer surplus males over other females because feeding competition between the sexes is assumed to be less severe than among females due to the higher reproductive and energetic constraints imposed on females compared to males (Wrangham 1980; Jolly 1984; Vasey 2000). Several possible benefits for females from surplus males have been proposed: females may benefit from decreased predation risk due to male vigilance (van Schaik



and Hörstermann 1994), from decreased risk of infanticide if males protect infants (Sommer 1994) or from increased social thermoregulation (Morland 1993; Overdorff 1998).

The aim of this study was to evaluate the latter hypothesis in redfronted lemurs. Results from a previous study on the same population revealed that in each group one male could be classified as dominant, monopolising social interactions with females and being the winner in all conflicts with co-resident males and females, while all other males were defined as subordinates (Ostner and Kappeler 1999). Conflicts between subordinate males and females were not as clear-cut as expressed in a high rate of undecided conflicts (Ostner and Kappeler 1999). In this study two hypotheses were examined: (1) redfronted lemurs face cold stress during phases of low temperatures and (2) females gain in terms of social thermoregulation from the presence of surplus males in their groups. The following predictions were tested: (1) animals will respond to cold temperatures by increasing the time spent inactive at minimum temperatures (below 15°C) compared to moderate temperatures and (2) subordinate males will participate more frequently in huddling groups with females than in subgroups composed of males alone.

## METHODS

The study was conducted in Kirindy Forest, a dry deciduous forest located in Western Madagascar within a forestry concession operated by the Centre Formation Professionnelle Forestière de Morondava. The area is characterized by pronounced seasonal changes with a hot rainy season from November to March/April (mean annual rainfall 787mm) and a cool dry season between May and October (Sorg and Rohner 1996). During the study daily minimum and maximum temperatures were recorded with mean maxima and minima of 33.7°C and 18.0°C, respectively.

Tab. 1: Composition of study groups and number of observations

group	adult males	adult females	juveniles/infants	morning observations*
A	4-5	2-3	3	89 (31)
B	3-4	3	3-4	91 (21)
F	3	2	3	50 (8)
J	2	2-3	1-2	44 (22)

\* in brackets: number of observations at morning temperatures below 15°C

Data were collected on four multimale-multifemale groups of redfronted lemurs at the time of daily minimum temperature (shortly before sunrise) from May 1999 to

June 2000, yielding a total of 274 morning observations. Groups comprised 2 - 3 adult females and 2 - 5 adult males (Tab.1). As this study is part of a long-term project started in 1996 the identity, age and dominance status of all animals are well-known (Ostner and Kappeler 1999). While resting in a huddling-group individuals could be identified easily by posture, scars and sexually-dimorphic pelage colour. Observations included the state of group activity (active or inactive), which was possible because activity was generally very closely synchronized among all group members with all animals becoming active within approximately the same 5 min period. Furthermore, the composition of huddling groups during cold morning temperatures (below 15°C) was recorded, including the identity of all participating animals. Out of the 274 morning observations 82 were conducted on cold mornings (below 15°C) during which data were collected on the composition of 204 huddling groups.

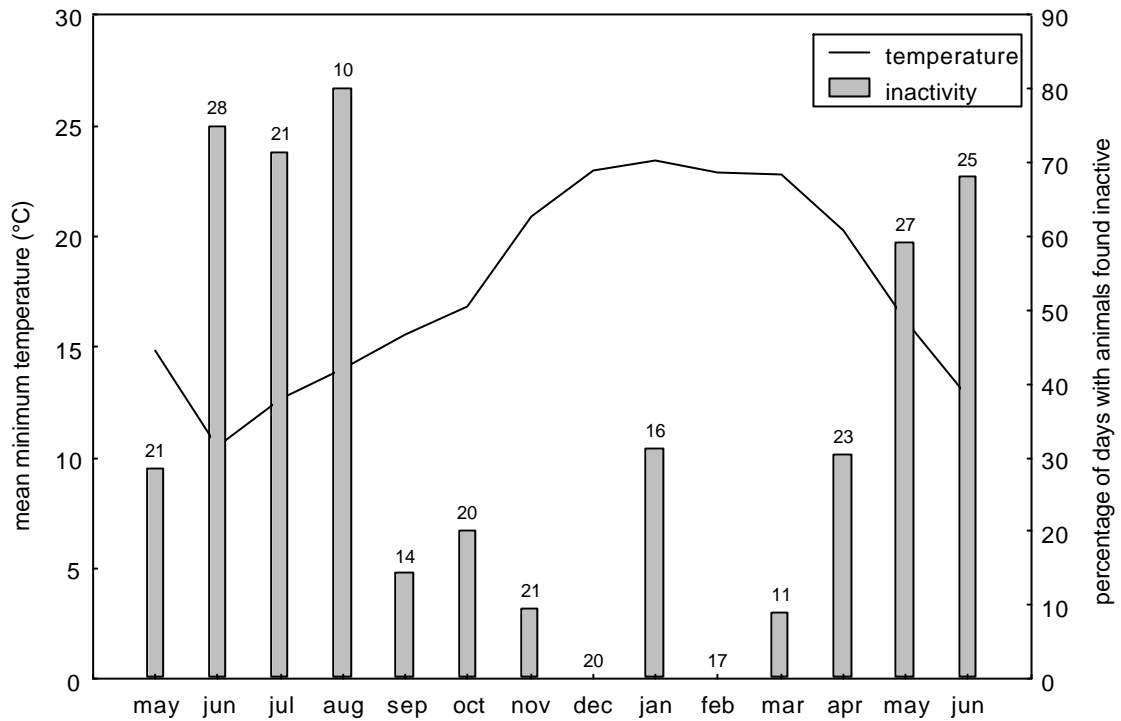
The relation between ambient temperature and state of activity was investigated with Spearman correlations. In order to evaluate whether subordinate males participated more frequently than expected in subgroups including at least one female, the number of subordinate males huddling with at least one female (regardless of the number of other subordinate or dominant males also participating in this subgroup) vs. the number of subordinate males huddling with males only, was calculated for each of the 82 morning observations. The difference in the overall frequency of subordinate membership in mixed versus male-only huddling groups was tested using a  $\chi^2$ -test.

## RESULTS

Animals reacted to low temperatures with inactivity. The monthly mean number of days with groups found inactive at sunrise correlated negatively with mean minimum temperature (Spearman correlation by ranks:  $n = 14$ ;  $r_s = -0.74$ ;  $p < 0.05$ ; Fig. 1). In all 82 observations with temperatures below 15°C animals formed huddling groups of various compositions, supporting the hypothesis of social thermoregulation. However, in 64.5% of these observations at least one subordinate male did not participate in a huddling-group with females. Subordinate males participated significantly more frequently in male-only groups than in huddling groups with females: female groups were joined by a subordinate male on only 40% of

occasions, in the remaining 60% subordinate males stayed with other males or were by themselves. ( $n = 204$ ;  $\chi^2 = 10.37$ ;  $p < 0.01$ ; Fig 2).

FIGURE 1: COMPARISON OF MONTHLY MEAN MINIMUM TEMPERATURE AND ACTIVITY AT



TIME OF DAILY MINIMUM TEMPERATURE OVER THE COURSE OF 14 MONTHS (5/99-6/00). NUMBERS ABOVE BARS INDICATE NUMBERS OF SAMPLED DAYS PER MONTH.

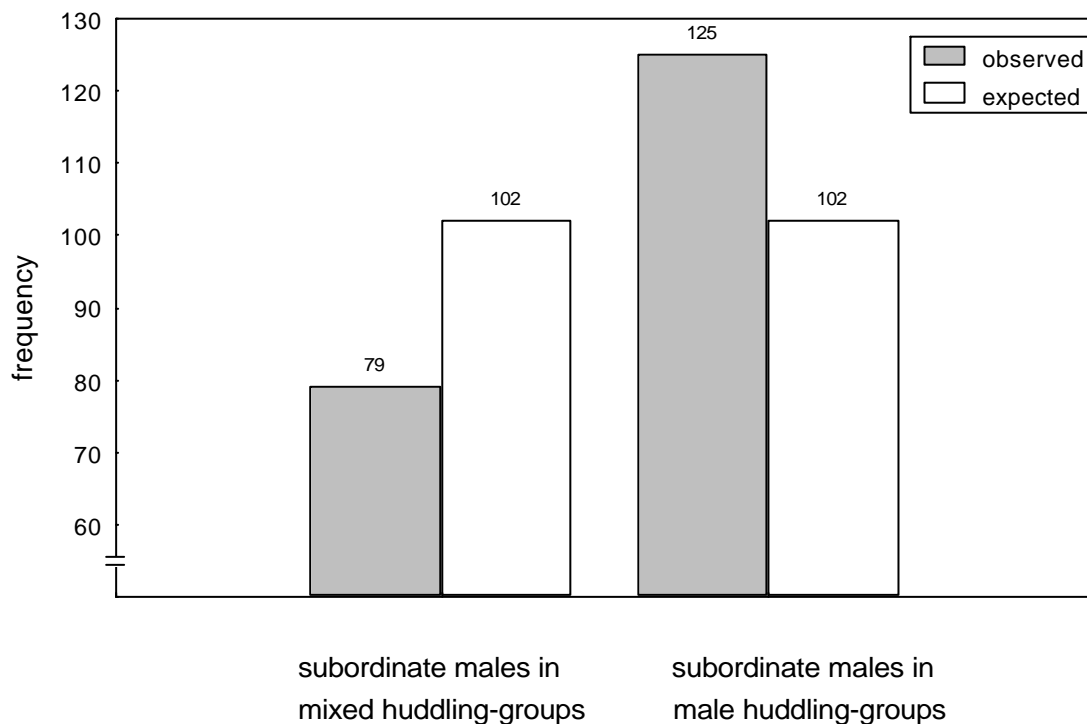


Figure 2: Observed and expected frequencies of participation of subordinate males in huddling groups including females versus in huddling groups with males alone.

## DISCUSSION

Redfronted lemurs apparently face cold stress during the cold months of the dry season and respond with increased inactivity and formation of huddling groups to low temperatures - probably to conserve energy. This result supports the hypothesis of social thermoregulation and is in accordance with findings from previous studies on the same species as well as on *Lemur catta* and the pair-living *Eulemur mongoz* (Tattersall 1976; Curtis et al. 1999; Donati et al. 1999; Pereira et al. 1999). Although in two of these studies (Curtis et al. 1999; Donati et al. 1999) nocturnal activity increased during the dry cold months, activity was concentrated in the first, warmer part of the night. Cold stress, however, is highest during the second half of the night with a peak of minimum temperature around the time of sunrise. Indeed, Donati et al. (1999) and Curtis et al. (1999) found in their studies an increase in inactivity at this critical time period. Although social thermoregulation seems to be a mechanism by which redfronted lemurs reduce cold stress, it fails to explain the unusual sex ratio, because surplus males did not participate more frequently than expected in huddling groups with females. Therefore, the hypothesis that females benefit from surplus males by increased social thermoregulation has to be rejected and there must be other determinants of the unusual sex ratio exhibited by these groups. Possible benefits for females from a high number of males include predator avoidance, infanticide avoidance and paternal care (Sommer 1994; van Schaik and Hörstermann 1994; summarised in Kappeler 1999a) and deserve further research.

## ABSTRACT

Pronounced seasonality with temperatures dropping as low as 5°C during the dry season has led to the hypothesis that Malagasy lemurs face cold stress and respond to this by inactivity and social thermoregulation, i.e. resting in tight body contact with conspecifics. As compared to anthropoids, lemur groups are comprised of an unusually high number of males, leading to an even or slightly male-biased adult sex ratio. According to one hypothesis, females may benefit from these surplus males in their groups if males huddle with females. The results of this study on redfronted lemurs (*Eulemur fulvus rufus*) in Kirindy Forest/Madagascar revealed that the animals indeed responded to cold ambient temperatures by increased inactivity

and the formation of huddling groups. However, surplus males did not participate more frequently than expected by chance in huddling groups with females and females do not, therefore, benefit from the high number of males in their groups by increased social thermoregulation.

## CHAPTER 4:

Male life history and the unusual adult sex ratios of redfronted lemur (*Eulemur fulvus rufus*) groups

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## INTRODUCTION

The illumination of the adaptive basis of variation in mammalian social systems has been a primary goal of socioecology (Crook and Gartlan 1966, Eisenberg et al. 1972; Rubenstein and Wrangham 1986). The distribution and association patterns of adults of both sexes constitutes the most basic aspect of any social system. While adult females distribute themselves according to the distribution of risks and resources in the environment, adult males respond primarily to the distribution of fertile females in space and time (Emlen and Oring 1977). Compared to most other orders of mammals, the diversity of primate societies and their variability within and among populations has been comparatively well documented and analysed (e.g., Smuts et al. 1987, Kappeler and van Schaik 2002). Among gregarious species, much of the variation in social organization among species and populations can be reduced to variation in the number of adult males within groups (Kappeler 2000a). This variation, which has far-reaching consequences for male and female reproductive strategies, has been causally linked to the temporal distribution of receptive females (Ridley 1986), female group size (Andelman 1986, Altmann 1990, Mitani et al. 1996a), the interaction of both factors (Nunn 1999) as well as to associated benefits for males and females (Kappeler 1999a).

In group-living species, theoretical considerations indicate the existence of a fundamental conflict of interests between the sexes over the adult sex ratio. Females may gain ecological as well as social benefits from a high number of co-resident males. For example, males in some species are better at detecting and repelling predators (van Schaik and van Noordwijk 1989) and may thus lower the overall risk of predation (van Schaik and Hörstermann 1994) apart from lowering per capita predation risk (Hamilton 1971). Moreover, females will only be able to exercise mate choice when more than one male is present. And finally, the rate of infanticide is often reduced in multi-male groups because single-male groups face a higher chance of group take-over and subsequent infanticide (Newton 1986, Watts 1989, but see Borries and Koenig 2000). To achieve these goals, females may form large groups, synchronize their fertile periods (Ims 1990) or they may transfer into a group with the optimal number of males (Sterck 1997, Steenbeek et al. 2000).

Males, on the other hand, should prefer to live in single-male groups, where they can monopolize reproduction, over membership in a multi-male group, where

they presumably have to share paternity among resident males (Kappeler 1999a). Under certain circumstances, however, it may also pay a male to tolerate additional males in his group (Kappeler 1999a). Such possible benefits include a decreased predation risk for themselves and their offspring (van Schaik and Hörstermann 1994), the promotion of the reproductive success of related males (e.g. Goldizen 1990; Pope 1990), and availability of support of male coalition partners against extra-group males (e.g. Wrangham 1979), the latter being especially effective if the coalitions consist of co-resident kin (Struhsaker and Pope 1991; Goldberg and Wrangham 1997).

Which sex ultimately controls adult group sex ratios is far from resolved, however. In this paper, we contribute important comparative information to the illumination of this conflict between the sexes from a group of primates with unusual adult sex ratios.

Within this socioecological framework the gregarious lemurs of Madagascar (Lemuriformes) constitute a paradoxon. They live in relatively small groups with only few adult females (Kappeler and Heymann 1996) and highly variable adult sex ratios (Kappeler 2000c). Despite a lack of sexual dimorphism in body and canine size (Kappeler 1990; 1996), which is generally associated with reduced direct male-male competition (Harcourt et al. 1981; Plavcan et al. 1995; Plavcan and van Schaik 1997), lemur males can typically be arranged along a dominance hierarchy (Pereira and Kappeler 1997; Kraus et al. 1999; Ostner and Kappeler 1999, but see Richard 1992). Males disperse from their natal group, whereas females are generally philopatric (Sussman 1992; Richard et al. 1993; Wimmer and Kappeler 2002), although aggressive eviction of single females has been observed in some species (Vick and Pereira 1989). In anthropoids these characteristics would inevitably lead to the formation of single-male groups (Andelman 1986; Pope 2000). Harems (i.e. one-male – multi-female groups), however, are never the modal grouping pattern in gregarious lemurs; instead groups are characterized on average by an even or male-biased adult sex ratio (Kappeler 2000c).

Redfronted lemurs (*Eulemur fulvus rufus*) exhibit this combination of idiosyncratic lemur traits. These 2 kg cathemeral primates live in groups of 5 - 12 individuals with an even or male-biased sex ratio (Sussman 1974; Overdorff 1998; Overdorff et al. 1999; Wimmer and Kappeler 2002). Genetic analyses indicated that closely related co-resident females form the core of stable groups whereas males



typically migrate (Merenlender 1993; Wimmer and Kappeler 2002). In wild populations either functional one-male groups were found with a dominant male monopolising social and sexual interactions with all females (Ostner and Kappeler 1999) or evidence of clear dominance relations among co-resident males was lacking altogether (Overdorff 1998; Gerson 2000). Despite the lack of sexual dimorphism in body and canine size, redfronted lemur males engage in aggressive interactions over access to receptive females during the mating season (Overdorff 1998; Ostner and Kappeler 1999). Preliminary data on reproductive success of wild redfronted lemur males revealed that the dominant male indeed fathered at least 66% of the offspring (Wimmer and Kappeler 2002). Thus, monopolization of access to several females is apparently possible for males at the behavioral level, but not at the demographic level. The question therefore arises, why redfronted lemur groups contain so many males and, more generally, who controls group composition?

To begin answering these questions, we examine i) the proximate determinants of the unusual sex ratio in redfronted lemur groups, ii) the temporal distribution of female fertile phases within groups, iii) variation in the adult sex ratio and iv) possible social benefits of the high number of males for both sexes. Taken together, this integrative approach will also provide an implicit test of the generality of the socioecological model for the evolution of primate social organization.

## METHODS

### **Study site and population**

The 60 ha study area is located in Kirindy forest, 60 km northeast of Morondava, western Madagascar, within a forestry concession of the Centre Formation Professionnelle Forestière de Morondava and part of the field station of the German Primate Centre (DPZ). For a detailed description of the dry deciduous forest see Ganzhorn and Sorg (1996). Between 1996 and 2002, 98 individuals from 7 groups (A, B, D, F, H, I, J) have been captured and individually marked with radio collars (Biotrack, Wareham, Dorset, UK) or colored nylon collars during brief anaesthesia (induced by applying 0.2 ml GMII; Rensing 1999) after blow-pipe darting by an experienced Malagasy technician. Only adults without dependent infants were darted and radio collars were replaced every year.

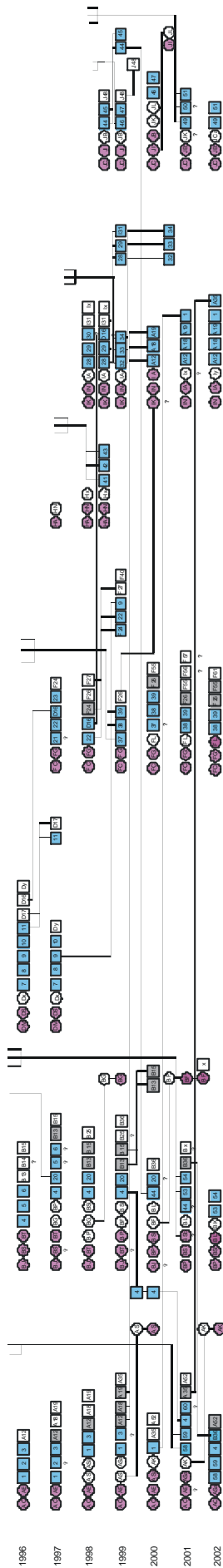


Figure 1: Group histories of redfronted lemurs in Kirindy Forest between 1996 and 2002. Circles: females; squares: males; coloured symbols: adult animals; grey squares: natal dispersal; female acronyms: group letter and first letter of name; male acronyms: letter of natal group if known and number; see appendix for full names used in previous publications on the same population; cross: dead; ? : fate unknown.

Animals were returned to their social group within 1 hour and we have not observed any injuries or adverse effect on behavior as a result of the capture procedure or the collars.

Group histories of these seven study groups are illustrated in Figure 1. Home ranges of five groups (A, B, F, I, J) lay within the study area. The range use of these five main study groups is depicted by 95% and 50% Kernel home ranges (Worton 1989), which represent the area with a 95%, respectively 50% probability to encounter the group (Fig. 2). Using the GIS animal movement software (Hooge & Eichenlaub 1997) home range size and range overlap were calculated. Home ranges were on average 19.3 ha (s.d. = 7.6) and overlapped considerably between groups ( $42.6 \pm 17.5\%$ ). Average home range size calculated as 95% Minimum Convex Polygons (Mohr 1947) was  $25.6 \pm 9.8$  ha and mean overlap was  $75.5 \pm 31.7\%$ . Outside our study area the number of individuals in different age-sex classes were determined opportunistically in 16 additional groups in the area.

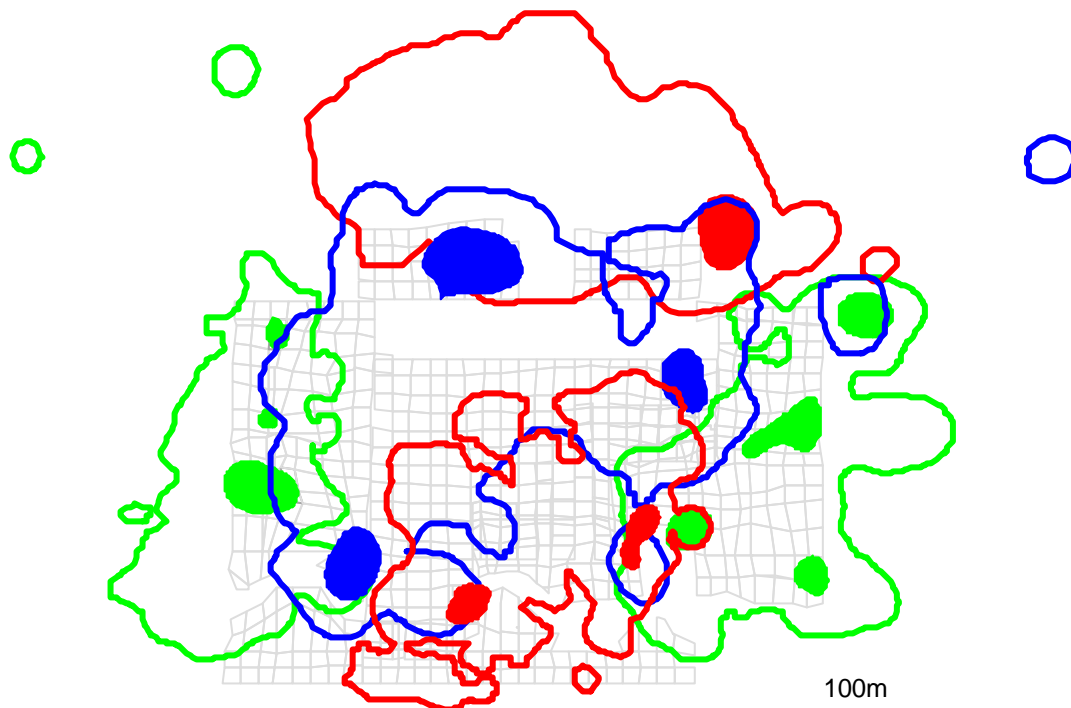


Figure 2: Kernel home ranges (95% probability and solid colours 50% probability) of five main study groups between April 1999 and July 2000 to illustrate the mutual range overlap among neighboring groups.

## Measures

Age at first reproduction among females in this population was three years, hence all animals were considered adult in their third mating season, i.e. at an age of 32 months (see also Izard et al. 1993, 1994). Animals with unknown birth dates were aged according to their body weight and dental wear by PMK (see also Richard et al. 1991). Birth rate in the main study groups, calculated as the number of births ( $n = 45$ ) divided by the number of “female-years” ( $n = 54$ ), was 0.83.

Descriptions of group size and adult sex ratio were based on data from all 23 groups. All demographic variables, such as migration rate, mortality, natal status of males and days of parturition were based on data recorded in the 8 main study groups during nearly daily censuses and/or observations by the authors and field assistants since 1996. Rate of infant mortality was inferred from the number of infants that disappeared before weaning (4 months, Ross and Jones 1999). Data on adult mortality were based on actual deaths. Only those cases entered the analyses where we had evidence from identifiable remains (collar, transponder in predator feces) for the death of the respective animal. Data on birth sex ratio include only those infants who survived long enough to be sexed, i.e., about 8 - 10 weeks, once the sexually dichromatic pelage pattern developed. Rate of migration was calculated using only those cases where animals were seen at least once after they left the group. Female and male group size were expressed as the average number of females or males in a group during the study period or the total number present at the time of a take-over. The terms “migration” and “dispersal” are used synonymously throughout this paper and refer to “circumstances in which individuals leave their existing home ranges and do not return, at least not in the short-term” (Stenseth and Lidicker 1992, p.5).

Redfronted lemur females show no visual sign of ovulation and ovarian function could not be measured non-invasively in the wild (Ostner and Heistermann in press). Therefore, the temporal distribution of fertile periods was determined by counting backwards from the day of parturition, using the average gestation length of 121 days (Izard et al. 1993). Based on a large sample ( $n = 33$ ) of gestation lengths in redfronted lemurs it has been suggested that females delivering infants at least 7 days apart are unlikely to have experienced synchronous estrouses (c.f. Pereira and McGlynn 1997).

## Statistical analyses

All statistical tests were applied two-tailed with an alpha level of  $p = 0.05$  and performed as described in Siegel and Castellan (1988). Descriptive statistics are reported as means and standard deviation (SD).

## RESULTS

First, we characterize the adult sex ratio in Kirindy redfronted lemur groups. The mean female groups size in 23 groups was 2.8 (SD = 0.81, range: 2 – 4), whereas male group size was 3.8 (SD = 1.11, range: 2 – 7). The ratio of adult females to males was 1 : 1.4. There was a significant positive correlation between male and female group size (Spearman correlation by rank:  $n = 23$ ,  $r_s = 0.532$ ,  $p = 0.009$ )

Adult sex ratio is influenced by several factors, such as sex ratio at birth, sex-biased mortality and migration. Of the 45 infants born between 1996 and 2001 in the main study groups, 17 female and 18 male infants survived long enough to be sexed. Overall infant mortality was 27%, but only five infants with known sex died, which precludes the analysis of sex-biased infant mortality. For future research, we developed a non-invasive tool to reliably assess fetal sex from maternal fecal samples (Ostner and Heistermann in press). Adult mortality rate per year was 7.6% for females and 10.8% for males.

## Migration

Both sexes migrated from their natal groups. Five of eleven females (45%) born and grown up in the study groups left their groups, four of them after several days of targeted aggression by co-resident females. These bouts of aggression took place around the time the respective females entered puberty (i.e., at 23 – 26 months). In all four cases aggression was directed against a daughter of a subordinate female by members of the dominant female's matriline. Females, that were evicted from their groups did not immigrate into another group but were observed to range solitarily for at least one year. In the fifth case a young female (14 months) left her natal group with her mother after the group had been taken over by unfamiliar males. This was the only observed case of a transfer of a fully adult female.

In contrast, 80% of males that became adult in the study population left their group (12 of 15), the remaining three males just reached adulthood at the end of the sampling period. The four youngest males to emigrate (4 – 25 months) migrated together with their likely fathers after their natal group was taken over by strange males. The other males left at an age of between 35 and 67 months. Redfronted lemur males are adult at 32 months and therefore some of these individuals delayed dispersal for a considerable amount of time. These 15 males who matured during the study period, delayed their dispersal for on average of 15 months, which corresponds to 1.2 mating seasons (SD = 1.1). Fully adult natal males are not expected to play an active role in the reproduction within their natal group. The corrected adult sex ratio that excludes this class of males was almost even with 1 F : 1.2 M.

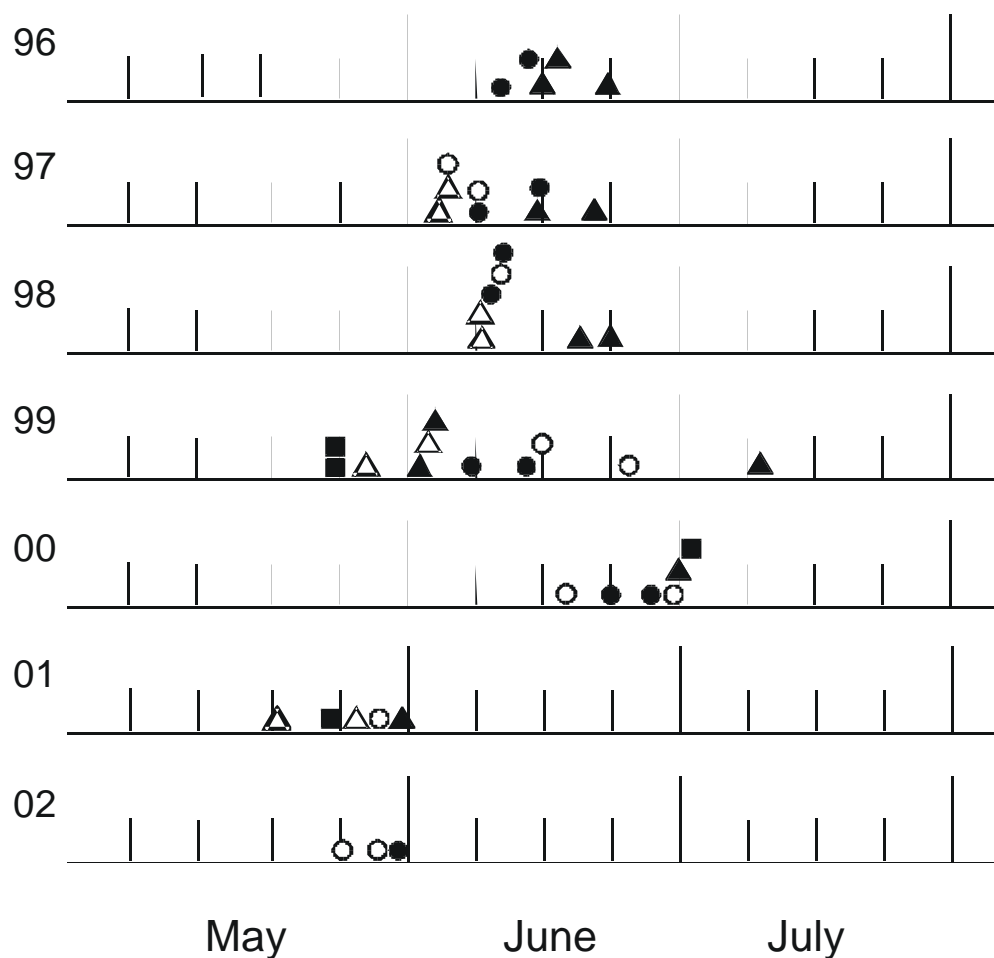


Figure 3: Temporal distribution of conceptions in five main study groups between 1996 and 2002. Each symbol represents day of conception, identical symbols indicate same group. Day of conception determined by counting backwards from the observed day of parturition. In 18 cases with multiple births per group, synchrony was likely in 14 cases.

Males migrated either alone or with one or two other males. We observed 13 cases of singly migrating males, and in three cases we have behavioral data on dominance relations among males following immigration. Whenever a single male immigrated into a group, he entered into a subordinate position and received no severe aggression from other group members, which closely resembles the migration pattern in another population of redfronted lemurs (Overdorff et al. 1999). On the other hand, in 9 cases involving 22 males, males migrated jointly. In six cases where we have information about the history of the target group, all former resident males left the group after immigration of new males.

### **Monopolization potential: female group size and estrous synchrony**

The number of females in the Kirindy population was low, ranging between 2 and 4 females, thus, theoretically allowing a single male to monopolize a group of females. However, the temporal distribution of fertile periods, calculated by counting backwards from the exact day of parturition, showed that estrous synchrony was likely in 14 of 18 cases with multiple births per group (Fig. 3).

### **Take-overs**

Six cases of group take-over by coalitions of two or three males were observed (Tab. 1). Resident males defended their group jointly while the challenging males also co-operated in expelling their opponents. Aggressive interactions continued for several days until all former resident males left the group, whereas the intruding males stayed. The probability of a take-over was independent of female group size, because the number of adult females at the time of take-over was not significantly higher than the mean number of females over the entire study period (binominal test:  $n = 6$ ;  $p = 0.656$ ). The ratio of adult females to males at the time of take-over was not more female-biased than the mean population sex ratio (binominal test:  $n = 6$ ;  $p = 0.656$ ). The probability of a take-over increased, however, in all cases with decreasing male group size, the probability that take-overs in groups with a few males occurred only by chance was small (binominal test:  $n = 6$ ;  $p < 0.05$ ). In other words, the more resident males were present to defend a group of females, the lower the probability of a take-over. In all six cases the number of intruders was higher than or equal to the number of resident males (binominal test:  $n = 6$ ;  $p < 0.05$ ).

Table 1: Demographic contexts of group take-overs

Group	Date	AF	AF at Take-Over	AM	AM at Take-Over	Intruding Males	Sex Ratio (F : M)	Sex Ratio at Take-Over
A	Jul 00	2.3	3	3.6	1	2	1 : 1.6	1 : 0.3
F	Feb 99	2.2	2	3.3	3	3	1 : 1.5	1 : 1.5
I	Dec 98	2.3	2	3.3	3	3	1 : 1.4	1 : 1.5
I	Jun 00	2.3	3	3.3	3	3	1 : 1.4	1 : 1.0
J	Mar 99	2.3	2	2.3	2	2	1 : 1.0	1 : 1.0
J	Nov 00	2.3	3	2.3	1	2	1 : 1.0	1 : 0.3

AF, AM: mean numbers of adult females respectively males during the study period; AF, AM at take-over: absolute number of adult females and males at the time of a take-over.

Twice we observed a one-male - multi-female constellation, due to the emigration of several natal males as well as the death of male group members. These harem situations lasted one and two months, respectively. Tenure in multi-male groups was considerably longer, averaging 29 months (SD = 16.81, range: 15 – 65, n = 11), which is an underestimation, because in many cases male group membership was either established when the study started or still continues.

Given that several groups of redfronted lemurs live in overlapping home ranges within the study area, migrating males should be able to assess the probability to successfully take-over a group, relative to the other available groups in the vicinity. In Table 2 the demographic parameters at the time a take-over occurred are given. Female group size was nearly constant in these cases (2 – 3), whereas the number of males ranged from 1 to 4 males. In 5 of 6 cases take-overs occurred in groups with the relatively smallest number of males available at this moment. Consequently, in 5 of 6 cases males took over those groups with the most female-biased sex ratio available (Table 3). These results therefore indicate that the number of males, rather than female group size, influences the rate of take-over.

Table 2: Group composition of the various study groups at the time of take-over.

Target group	Number of females						Number of males					
	A	B	F	H	I	J	A	B	F	H	I	J
A	3	3	2	n.d.	3	3	1	2	4	n.d.	3	2
F	2	2	2	2	2	n.d.	3	4	3	3	3	n.d.
I	2	2	2	2	2	n.d.	3	4	4	3	3	n.d.
I	3	3	2	n.d.	3	3	4	2	4	n.d.	3	2
J	2	3	2	2	2	2	3	4	3	3	3	2
J	3	2	2	n.d.	2	3	3	2	3	n.d.	3	1

Bold numbers indicate the target group of a take-over; n.d.: no data available



In three groups take-overs occurred during the gestation or lactation period, so that five infants were born after their fathers had left the group. In the case in which the new males entered the group during gestation (group A, July 2000), both infants disappeared four days after their birth. The other three infants disappeared within days (1 – 12) of the take-over. The mean age of infants at disappearance was 36 days (4 – 88). Therefore, all infants sired in the mating season preceding a take-over disappeared before weaning, indicating that a take-over dramatically increases infant mortality rate if compared to the general moderate rate of 27%.

Table 3: Sex ratio (F : M) variation among different groups at the time of take-overs.

Target group	Sex ratio Group A	Sex ratio Group B	Sex ratio Group F	Sex ratio Group H	Sex ratio Group I	Sex ratio Group J
A	<b>1 : 0.3</b>	1 : 0.7	1 : 2.0	n.d.	1 : 1.0	1 : 0.7
F	1 : 1.5	1 : 2.0	<b>1 : 1.5</b>	1 : 1.5	1 : 1.5	n.d.
I	1 : 1.5	1 : 2.0	1 : 2.0	1 : 1.5	<b>1 : 1.5</b>	n.d.
I	1 : 1.3	1 : 0.7	1 : 2.0	n.d.	<b>1 : 1.0</b>	1 : 0.7
J	1 : 1.5	1 : 1.5	1 : 1.5	1 : 1.5	1 : 1.5	<b>1 : 1.0</b>
J	1 : 1.0	1 : 1.5	1 : 1.5	n.d.	1 : 1.5	<b>1 : 0.3</b>

Bold numbers indicate the target group of a take-over; n.d.: no data available

## DISCUSSION

The results of this study confirmed that redfronted lemurs live in groups with a male-biased adult sex ratio. Our study suggests that certain life history traits may ultimately play a role in shaping redfronted lemur social organization and that this unusual adult sex ratio may provide benefits for both males and females, thereby considerably defusing the conflict of interests between the sexes.

### Proximate determinants of sex ratios

The observed surplus of males in a given group at a given time may result from either delayed male dispersal or female transfer, or both. Indeed, a considerable number of fully adult, natal males delayed their dispersal, and a high percentage (5 of 11) of juvenile females had left their natal groups before reaching adulthood. However, if only non-natal males are considered, the sex ratio is even and the question of the unusually high number of males remains. Thus, we need to explain why these lemur groups contain on average more, and in most cases considerably more, than one male.

At a proximate level, three demographic mechanisms could potentially modify adult sex ratios. Birth sex ratio and mortality rates for males and females were even or, in the case of adult mortality, male-biased, so that heavily sex-biased birth or mortality rates can provide no explanation. Dispersal, which is costly in terms of lost reproductive chances while migrating between groups as well as an increased predation risk during this solitary phase (Alberts and Altmann 1995), could contribute to even adult sex ratios. However, despite a relatively high rate of females exhibiting involuntary dispersal, males migrated even more frequently. Male migration in this population may not pose such high risks for males as for other primates, because home ranges overlap and males often disperse into neighboring groups, and because many males do often not migrate solitarily but with other males.

### **Determinants of monopolization potential**

An even adult sex ratio in the population does not explain why males do not expel each other from individual groups. According to a threshold of female group size for successful monopolization by a single male observed in cercopithecine primates (Andelmann 1986), the number of lemur females per group would lead us to predict a social organization consisting of single-male groups. Single-male groups were observed twice, but they lasted relatively briefly (1 and 2 months, respectively) before the respective groups were taken over by intruding males. The temporal distribution of female fertile phases indicated that the occurrence of estrous synchrony is possible. Moreover, the observation that copulations occurred over several weeks suggests that males were unable to detect the fertile period of a given female (Ostner and Kappeler 1999). Thus, at least from the males' perspective, females were synchronous, which lowers the monopolization potential of a single male and, hence, the probability that harem groups will form (Ridley 1986; Nunn 1999).

Another factor hampering the formation of harem groups may be the cathemeral activity pattern of redfronted lemurs, which is characterized by regular bursts of activity during the day and at night. Rowell (1988) suggested that good visibility increases harem holding potential. Visibility at night is reduced, making it difficult for a single male to guard more than one female. In our study population this effect is magnified because the proportion and duration of nocturnal activity

increased during the mating season (Donati et al. 1999; Kappeler and Erkert submitted ms).

### **Social benefits for males**

The probability of the occurrence of a take-over was assessed using two different methods. Comparing group composition at the time of take-over with the average composition for the respective group, we found that the number of resident males was always lower at the time of take-over than the mean number of males. In addition, the number of intruding males was always higher or at least the same as the number of resident males, indicating that larger coalitions of intruding males have a higher chance of taking over a group. Because all males participate either in group defence or in expelling resident males, resident males benefit from a large number of co-resident males. Similarly, immigrating males benefit from forming coalitions. This analysis, however, does not consider the possibility that migrating males may have several groups to choose from. Therefore, we compared the group composition of the actual target group of a take-over with all other available groups at that moment. The number of females was not decisive for the chance of a take-over because female group size varied only little among the different groups. Take-over occurred mainly in those groups with a relatively small male group size and a female-biased sex ratio, again indicating that the number of males per group is a crucial factor for the probability of a take-over. An obvious shortcoming of this method is that we do not have information on all potential available groups at a given time. However, both analyses produced the same factor, i.e. male group size, as a main predictor of take-over rate.

A low harem holding potential is further suggested by the observation that dominant males never expelled their coalition partners after a successful take-over, as do most Hanuman langurs, *Semnopithecus entellus* (Sommer 1994), for example. Dominant males do not suppress testicular function of subordinate males, unlike other primates living in multi-male groups (Schilling et al. 1984; Wickings and Dixson 1992; Brockman et al. 1998) and even tolerate their mating activity (Ostner and Kappeler 1999; Ostner et al. 2002), indicating that dominant males benefit from the presence of additional subordinate males in their groups, whereas the benefits for subordinate males are less obvious.

Jointly migrating males were not necessarily closely related. Based on results of a study on the genetic relationships within this population (Wimmer and Kappeler 2002), it was apparent that some coalition members shared the same mitochondrial haplotype, whereas others did not. Kinship, however, is not a necessary precondition for successful male coalitions. A well-known example of male cooperation among related as well as non-related males is provided by lions, *Panthera leo* (Bygott et al. 1979; Pusey and Packer 1987). Male lions either form coalitions of natal males that disperse together and enter a new pride, or one or two males team up with single males from other prides before gaining residency (Packer et al. 1991). As in redfronted lemurs, the probability of taking over a pride increases with increasing coalition size in lions (Packer et al. 1988). Larger coalitions, however, contain fewer non-related males, which has been attributed to the increasing within-coalition variance in reproductive success as coalitions grow larger (Packer et al. 1991). If there are non-breeding helpers in a coalition, they can only benefit in terms of inclusive fitness if they are related with the breeding males. Cooperation between non-relatives on the other hand is most likely when all group members can breed (Packer et al. 1991; Johnstone 2000). Preliminary data on male reproductive success in redfronted lemurs revealed that non-related males shared paternity in two groups (Wimmer and Kappeler 2002), thereby providing subordinate males with an incentive to stay in the groups.

Among primates, red howler monkey, *Alouatta seniculus*, groups exhibit a very similar pattern to that of redfronted lemurs. Group size is relatively small and the majority of groups are multi-male, although single-male groups also exist (Pope 1990; Crockett and Janson 2000). Coalitions of males gain access to groups of females, but in contrast to redfronted lemurs, only the dominant male breeds (Pope 1990). Therefore, coalitions often consist of relatives, which last significantly longer than those of unrelated males (Pope 1990). After take-overs, bouts of infanticide frequently occur (Crockett and Sekulic 1984; Crockett and Janson 2000). In red howlers and some other primate species the frequency of male incursions was associated with an increased female group size (Borries and Koenig 2000; Crockett and Janson 2000), which is not mirrored by the results of this study, where take-overs were not more prevalent in groups with more females. The reason for this may be the extremely constrained female group size in redfronted lemurs, which is regulated by female targeted aggression directed against daughters of subordinate

females (Vick and Pereira 1987; see also Pereira 1995). Whether ecological constraints (Janson 1988; van Schaik 1989; Janson and Goldsmith 1995) or the risk of infanticide (Crockett and Janson 2000; Steenbeek and van Schaik 2001) limit female group size in redfronted lemurs has to be determined by future studies.

### **Social benefits for females**

Females may benefit in multiple ways from a relatively high number of males. Because the risk of a take-over is associated with male group size and take-overs pose an increased risk of subsequent infanticide, females gain by the presence of additional males in terms of better defence against intruding males. Infanticide by extra-troop males has been observed in *Eulemur fulvus* both in captivity and the wild (Jolly et al. 2000) and our data also strongly suggest the occurrence of infanticide following take-over by immigrating males. When a takeover occurred during the mating season females were observed to mate with all males present (JO, unpubl. data), which may be interpreted as a female anti-infanticidal strategy in which paternity is confused to some extent. If the rate of take-over and subsequent infanticide decreases with increasing male group size (e.g. Newton 1986; Robbins 1995; this study), estrous synchrony may be one mechanism with which females may influence the number of co-resident males. Additional possible benefits have been suggested, such as increased thermoregulation benefits and a reduced predation risk. While thermoregulation does not seem to rely on surplus males in this species (Ostner 2002), results of a study on vigilance behavior on the same population revealed that males were more vigilant than females (Hilgartner 2001).

### **Lemur life history and social organization**

Despite possible proximate explanations for the prevalence of multi-male groups, i.e. female reproductive synchrony and low monopolizability due to cathemeral activity, and the fact that resident males gain by additional males in their groups by communal defence against intruding males the question remains why this evolutionary route has been taken in lemurs, but not in anthropoid primates living in similarly small groups. Most lemurs deviate from anthropoids in that they exhibit a relatively fast life history with short interbirth-intervals and fast maturation (Kappeler 1996; Godfrey et al. 2001; Lee and Kappeler in press; but see Richard et al. 2002 and below) and this combination of life history traits may ultimately contribute to the

rareness of single-male groups. Young natal males benefit in terms of inclusive fitness if their father's tenure is long. They will, however, also gain from the possibility of forming coalitions with male group members with whom they can migrate together and gain residency in another group. A fast life history makes waiting for allies to grow up possible. Due to the lack of all-male-bands in redfronted lemurs, male allies can only be found within bisexual groups. Therefore, it may pay young, natal males to delay their dispersal and wait for allies to grow up (see also van Noordwijk and van Schaik 2001).

To further evaluate the relationship between life history traits and monopolization potential we reviewed life history parameters of 35 harem-living primate species from 10 genera. Most species exhibit an interbirth-interval considerably longer than redfronted lemurs, but some *Cercopithecus* species, as well as patas monkey, *Erythrocebus patas*, breed also on average every year (Lee 1999; Ross and Jones 1999). Time of first reproduction, however, is relatively long in the *Cercopithecus* species (Cords 1988; Ross and Jones 1999) and generally longer than mean tenure (Cords 1988), implying that following a take-over young males have to leave their natal groups before reaching adulthood. Mortality rates increase generally after emigration from the natal group (Rajpurohit and Sommer 1991; Alberts and Altmann 1995; Rajpurohit et al. 1995), precluding alliance formation with related males or males of the natal group, with whom to take-over a group. Only patas monkeys exhibit a similar combination of short interbirth-intervals and fast maturation and, thus, seem to contradict our argument. However, the social organization of patas monkeys and some closely related *Cercopithecus* species is unusual and despite a general single-male structure characterized by the regular influx of extra-group males and promiscuous mating (Chism and Rowell 1986; Cords 1988).

This hypothesis may not explain the even sex ratios characteristic of all gregarious lemurs because group-living in lemurs evolved twice in independent lineages (Kappeler 1999b; see also Ridley 1986). Only the members of the Lemuridae (*Eulemur*, *Lemur*, *Haplemur* and *Varecia*) appear to exhibit a relatively fast life history, lack of all-male bands and joint migration. For example, ringtailed lemur (*Lemur catta*) males also migrate in groups of two to three, leaving male-biased groups (Sussman 1992) and transferring into groups with fewer males (Jones 1983). However, the group-living members of the Indriidae family – the sifakas

(*Propithecus* spp.) – exhibit longer interbirth-intervals, reproduce much later and have been characterized by a generally slowed-down life history (Wright 1995; Richard et al. 2002). Joint migrations have also not been reported so far (Richard et al. 1993). Thus, relatively fast life histories may facilitate joint male dispersal, and thus contribute to the lack of harem groups, in true lemurs. Other, perhaps Madagascar-specific ecological factors caused by the harsh and unpredictable island environment (see Wright 1999), must also be involved because these unusual sex ratios characterize the members of two independent lineages (Kappeler 1999b).

## Conclusions

This study suggested that certain life history traits in males may be partly responsible for even sex ratios and the lack of single-male groups in redfronted lemurs. Male monopolization potential may be reduced if coalitions of young males challenge resident males to take-over bisexual groups whenever the number of males defending the group is low. Because the rate of take-over is directly correlated with the risk of infanticide, males and females share the common interest of living in groups with a relatively high number of males. This common interest may defuse intersexual conflict over group composition. If confirmed by other studies, this insight would lead to an extension of the socioecological model in that the distribution of males, and not only that of females, can influence male transfer decisions and thus group size and composition.

## ABSTRACT

In group-living species, theoretical considerations indicate the existence of a fundamental conflict of interests between the sexes over the adult sex ratio within groups. Females may derive certain benefits, such as decreased predation risk, opportunities for mate choice and infanticide avoidance, from living with many males. Males, in contrast, should generally try to monopolize access to a group of females. Which sex ultimately controls adult group sex ratio is poorly known. In this paper, we examine this conflict between the sexes in wild redfronted lemurs (*Eulemur fulvus rufus*), Malagasy primates characterized by an unusual lack of female-biased adult sex ratios. Using various demographic and behavioral data from several groups collected over six years, we examine (i) the proximate determinants of this unusual

sex ratio, ii) the temporal distribution of female fertile phases within groups as a determinant of male monopolization potential, iii) sources of between-group variation in the adult sex ratio, and iv) possible social benefits of the relatively high number of males for both sexes. Results of this study revealed that birth and mortality rates were not sex-biased and that males migrated considerably more frequently than females, providing no proximate explanation for the unusual sex ratio. However, certain life history traits (fast maturation, short inter-birth intervals) may ultimately play a role because they act to facilitate joint group transfers of male coalitions. Despite a relatively small female group size and an associated high monopolization potential, female estrous synchrony may prevent the formation of single-male groups. Reduced male group size seems to be the main predictor of take-over rate, and thus, infanticide risk, suggesting that both sexes may benefit from the high number of co-resident males, thereby considerably defusing the conflict of interests between the sexes.



# GENERAL DISCUSSION

The number of males in primate groups has far-reaching consequences for sex-specific reproductive strategies (e.g. Davies 1993) and is presumed to cause a fundamental conflict of interests between the sexes. Sexual selection theory predicts that males will compete for exclusive access to a group of females, which will lead to sexual dimorphism and a female-biased adult sex ratio within groups. If male monopolization potential is high, the modal grouping pattern will be single-male groups. Females may counteract male monopolization because they may gain social and ecological benefits from living in a multi-male group (Kappeler 1999a; 2000c). Proximately, females may regulate male monopolization potential, and hence the number of males within a group, by the degree of overlap in their fertile periods. In the first part of this discussion I will summarise the results of the present thesis on sex-specific reproductive strategies of redfronted lemurs. In the second part, I will discuss the findings in the light of hypotheses that aim to explain the lack of convergence between lemurs and anthropoid primates in social, demographic and morphological features.

## **Sex-specific reproductive strategies of redfronted lemurs**

Analyses of proximate aspects of reproductive strategies depend on reliable information about endocrine mechanisms and reproductive physiology underlying hormone-behavior interactions. In wild populations such information can only be gained by the application of non-invasive hormone measurements using urine or fecal samples. Because information on the feasibility of non-invasive hormone measurements in this species was lacking, I conducted the first study of male and female reproductive endocrinology in redfronted lemurs.

The results presented in Chapter 1 revealed that monitoring ovarian function is principally possible in this species, as demonstrated by the analysis of samples from captive females. Characterisation of ovarian cycles or determination of the day of ovulation in wild females was not feasible, however, because of high day-to-day variation in measured hormone levels. The high variation may have been caused partly by the storage procedure because it has recently been shown that storage in ethanol for longer periods may alter hormone concentrations (Khan et al. 2002). Another possibility for the variation are dietary factors. Redfronted lemurs are

important seed dispersers (Ganzhorn et al. 1999) and their diet consists mainly of fruits and seeds (Overdorff 1993), a large proportion of which is excreted in undigested form. Because dietary fiber composition is known to affect gastrointestinal transit time and fecal hormone concentrations (Wasser et al. 1993), the high and variable proportion of undigested fecal matter probably increased the variation in excreted hormone concentrations and resulted in variable and uninformative hormone profiles (c.f. Curtis et al. 2000). This assumption is supported by the finding that in two captive females, which fed on a much more digestible diet, distinct ovarian cycles could be distinguished. If these methodological problems indeed relate to specifics of feeding ecology of redfronted lemurs, they may not be solved easily in subsequent studies.

Despite these shortcomings in the evaluation of ovarian cycles in wild females, the study provided important first insights into the reproductive physiology of redfronted lemurs, such as information on endocrine changes associated with gestation and reliable calculations of gestation and cycle length (Chapter 1). Estrogen excretion during the second half of pregnancy was strongly related to the sex of the offspring born, with only females giving birth to male infants showing elevated estrogens levels. This observation allows reliable prenatal sex determination, which has broad implications for future studies of the reproductive biology of the species. For example, it allows reliable assessment of the sex ratio at birth, which up to now was impossible to determine because infants are sexually monomorphic for the first 2 - 3 months of age, a time during which a high proportion of infants has already died. The possibility of sexing infants prenatally opens the possibility to test hypotheses concerning sex ratio theory (e.g. Clutton-Brock and Iason 1986) and to collect data on sex-specific infant mortality in the first months of life.

Moreover, an estrogen rise based on fetal sex has not been observed in any non-lemur strepsirrhine or haplorrhine primate. It may represent a unique reproductive characteristic of lemurs, possibly linked to the expression of female genital masculinization and female dominance characterizing most lemurs (Ostner et al. submitted ms). More data on other lemur and non-lemur strepsirrhines are now needed to confirm this assumption and to assess the phylogenetic origin of this trait and its adaptive significance.

Due to the impossibility to characterize day of ovulation by endocrine measurements, synchrony of fertile periods could only be inferred indirectly. Using long-term records of demographic data from several groups, the degree of synchrony was assessed indirectly by analysing the temporal distribution of births within groups, taking the range of variation of gestation length into account (see Pereira and McGlynn 1997; Chapter 4). While this approach obviously cannot provide a definite answer to the question of reproductive synchrony, it represents an improvement over the previous method of using seasonality as an approximation of synchrony (Ridley 1986; Mitani et al 1996a). In about 80% (14 of 18) of cases, females were likely to have experienced estrous synchrony. This result indicates a proximate mechanism by which females may regulate the number of males in their groups, because a single male will be less capable to monopolize several females that are simultaneously receptive. The lack of single-male groups in redfronted lemurs in Kirindy Forest may therefore be at least partly and proximately attributable to the temporal distribution of receptive females.

The lack of sexual dimorphism in body size and weaponry is unexpected in the observed multi-male social organization because sexual selection theory predicts male competition for access to females. Behavioral data on agonistic relationships and dominance relations among redfronted lemur males are highly variable among studies. Studies in captivity revealed infrequent agonistic interactions, a high rate of undecided conflicts and in general no pronounced dominance relations (Kappeler 1993; Pereira and Kappeler 1997), which is in accordance with the expected low intensity and frequency of direct male competition inferred from the lack of sexual dimorphism. However, in a previous study of two wild groups during one mating season, males engaged in frequent, mainly decided conflicts and could be arranged along a linear dominance hierarchy (Ostner and Kappeler 1999). These results were reconfirmed in the present study by revealing an anthropoid-like social structure with a central, dominant male and a linear dominance hierarchy in each of four groups (Chapter 2). While this social structure is congruent with the theoretically expected high level of direct male competition, the question arises why the expected corresponding morphological features, i.e. sexual dimorphism in body and canine size, did not evolve at the same time. I will return to this point later.

In addition to direct competition for access to females males living in multi-male groups may compete indirectly for fertilizations. In Chapter 2 I investigated

whether redfronted lemurs males rely on an indirect form of intrasexual postcopulatory competition, such as endocrine suppression of testicular function (e.g. Keverne et al. 1982). Analysis of androgen levels in fecal samples revealed no difference between dominant and subordinate males, indicating that dominant males did not suppress the testicular function, i.e. sperm production, of subordinate rivals. Another mechanism of postcopulatory competition is sperm competition, which occurs whenever the sperm from two or more males compete to fertilize the egg(s) of a female during one reproductive cycle (Birkhead and Møller 1992). The large testes size found in redfronted lemurs males relative to other prosimians (Kappeler 1997) and the high frequency of matings observed (Overdorff 1998; Ostner and Kappeler 1999; Chapter 2) suggest that males compete intensely on the basis of their sperm. Reliance on an indirect mechanism of intrasexual competition helps to explain the lack of sexual dimorphism in redfronted lemurs. As noted above, however, male intrasexual competition takes also the form of direct aggressive combat. Because all males copulated, but overall mating success and paternities were skewed towards the dominant male (Ostner and Kappeler 1999; Wimmer and Kappeler 2002) I propose that the dominant male copulates at the best possible time in relation to ovulation giving his sperm an advantage over those of his rivals. Whether the dominant male's optimal timing is a function of his higher mating frequency or whether females choose the dominant male as mate at the time with the highest chance of fertilization can only be evaluate in an experimental setting.

### **Benefits of a high number of males**

If females regulate the number of males in their group, it is expected that they gain benefits from co-resident males. Several possible non-exclusive benefits for females from a high number of males have been proposed which can be distinguished on two levels. There are benefits derived directly from *living* with many males and those gained by *mating* with many males.

In groups with more males the risk of predation will be lower due to **dilution** effects (Hamilton 1971; Dehn 1990) and improved **vigilance** (van Schaik and van Noordwijk 1989; van Schaik and Hörstermann 1994; Koenig 1998). Adding a group member will decrease the chance of being caught by a predator simply by increasing the per capita risk of being chosen as prey (Dehn 1990). Studies of vigilance behavior in the Kirindy population indicated that females benefit from additional

males in their groups because male redfronted lemurs are more vigilant than females (Hilgartner 2001; Ostner 2001; but see Overdorff 1998). Moreover, females spent less time being vigilant themselves if more animals were present in their close vicinity, which allowed females to allocate time to feeding or social behavior (Ostner unpubl. data).

Each additional group member may improve **thermoregulation** if animals rest in close body contact with a conspecific (“huddling”) in order to save energy during cold periods (Morland 1993; Overdorff 1998). This proposed benefit was tested for the first time in this study (Chapter 3). The results revealed that redfronted lemurs reacted to low temperatures with an increase in time spent inactive and resting in huddling groups. However, subordinate males did not participate more frequently than expected by chance in huddling groups comprising females. Thus, the hypothesis of improved thermoregulation as a female benefit of a high number of males was not supported.

**Infanticide** is a major source for infant mortality (e.g. Sommer 1994; Borries and Koenig 2000; Crockett and Janson 2000) and the reproductive success of females can be greatly increased by reducing infanticide rate. If many males are excluded from breeding, as e.g. in populations comprised of single-male groups, intruder pressure will be high and the rate of breeding male replacement and ultimately that of infanticide will increase (Janson and van Schaik 2000). Results of this study suggest that the occurrence of a **take-over** by new males increases the rate of infanticide in redfronted lemurs as indicated by the increase in infant mortality following a take-over (Chapter 4). The rate of male replacement, in turn, was associated with the number of resident males with a small male group size being the main predictor of take-overs. A high number of males therefore decreases the rate of infanticide and improves female reproductive success (and that of siring males). At the same time, lower take-over rates prolong male tenure and thus increase the reproductive success of resident males (see Altmann 2000). Due to the close association of both male replacement and infanticide, females as well as males benefit from a high number of males in their group.

Last but not least the opportunity for **polyandrous matings** is a benefit females derive from living in a multi-male group. Mating with several males may increase females’ reproductive success if **paternal care** increases the survival of their offspring. If paternal care depends on the chance that a male has sired the

offspring, i.e. mated with the female, polyandrously mating females will receive the help of all males they copulated with, whereas a monogamous female will only have one helper (Davies et al. 1996; Davies 2000). However, redfronted lemurs males do not provide direct paternal care (Overdorff 1998; pers. observation) suggesting that increased paternal investment by several males is not a decisive benefit in this species. **Genetic benefits** can also be gained by mating with multiple partners (review in Jennions and Petrie 2000; Paul 2002). For example, polyandry decreases the risk of being inseminated by genetically incompatible sperm (Zeh and Zeh 1996; 1997). Generally females in multi-male groups have more mating partners to choose from, allowing a female to choose a male that complements her own set of genes, increasing heterozygosity which generally is beneficial for an individual (Brown 1997). Female redfronted lemurs have at least the chance to exert mate choice because they live with many males and because they are not absolutely restricted in their mating activity. Studies in different populations revealed that females mate polyandrously with all males in the group (Overdorff 1998; Ostner and Kappeler 1999). Whether females derive genetic benefits from reproducing with certain males is beyond the scope of this thesis. Finally, polyandry may be a counter-strategy against **infanticide** by co-resident males (Hrdy 1977; 1979). If infanticide is an adaptive male strategy (Hrdy and Hausfater 1984) and males do not kill their own offspring (Borries et al. 1999b), females can effectively reduce the risk of infanticide by confusion of paternity (Hrdy 1977; 1979; van Schaik et al. 1999; van Noordwijk and van Schaik 2000). By mating with all males in their groups female redfronted lemurs decrease the risk of infanticide by co-resident males.

In conclusion, female redfronted lemurs gain by a decreased risks of predation and infanticide as well as by the opportunity to exert mate choice from living and mating with many males. Because take-over rate decreased with an increasing male groups size resident males in this study also profited by living in multi-male groups, thereby defusing the conflict of interests between the sexes.

### **Male life history**

My studies suggest that female redfronted lemurs pursue reproductive strategies to regulate group composition. By synchronizing their fertile periods, females effectively lower male monopolization potential. However, certain life history characteristics of redfronted lemur males may additionally hamper the formation of

single-male groups (Chapter 4). A comparison of certain life history traits (interbirth interval, time to maturation) of primate species living predominately in single-male groups with those of redfronted lemurs revealed that the life history of redfronted lemurs is relatively accelerated. Patterns of migration and group take-over revealed that males can only successfully take-over a group of females if they form a coalition with other males. A fast life history allows young natal males to wait for allies to grow up. I therefore propose that the combination of life history traits may be partly responsible for even sex ratios and the lack of single-male groups in this species and possibly other members of the Lemuridae (*Eulemur*, *Hapalemur*, *Lemur*, *Varecia*), which exhibit a similar life history and show also joint male migrations.

However, this hypothesis does not provide an explanation for the unusual group composition among the gregarious species of the Indriidae (*Propithecus* spec.) because these species exhibit a relatively slow life history (Wright 1995; Richard et al. 2002) and lack joint take-overs (Richard et al. 1993). An unusual group composition is just one of several traits contrasting all gregarious lemurs from anthropoid primates. Because true lemurs (family Lemuridae) and the *Propithecus* species represent two independent group-living lineages (Kappeler 1999b; see also Ridley 1986), other factors must also be involved in the evolution of this unique combination of traits. Hypotheses explaining the evolution of many or all of these idiosyncratic traits may therefore be superior to the specific hypothesis developed in this thesis. These more general hypotheses will therefore be investigated in the following.

### **Evolution of lemur idiosyncrasies**

Lemur idiosyncrasies include social, demographic, morphological and ecological traits in which they deviate from anthropoid primates. In many lemur species (1) males are socially subordinate to females (Jolly 1966; Jolly 1984; Richard 1987; Pereira et al. 1990), (2) groups are relatively small and group composition is characterized by an on average even adult sex ratio (Kappeler 2000c), (3) lemurs lack sexual dimorphism in body and canine size (Kappeler 1990; 1996), (4) exhibit tight breeding seasonality (Hrady and Whitten 1987) and (5) cathemeral activity is relatively widespread (Tattersall 1987; Enquist and Richard 1991). Wright (1999) added (6) female targeted aggression, (7) abundance of pair-living species, (8) direct male competition and sperm competition, (9) high infant mortality and (10) metabolic

factors, i.e. low basal metabolic rate and torpor, to the above mentioned traits. Four not necessarily exclusive hypotheses have been proposed to explain the evolution of some or all of these unusual features.

### 1. Energy conservation hypothesis (originally Jolly 1966; 1984)

The “energy conservation hypothesis” (sensu Wright 1999) was first proposed by Jolly (1966) to explain the evolution of female dominance and has since been expanded. The hypothesis proposes that Madagascar’s extreme seasonality in climate and resource availability tightly restricts the period favourable for reproduction (Jolly 1984; Richard 1987). In addition, the relatively low basal metabolic rates found in lemurs lead to constraints on reproductive energetics (Pollock 1989; Young et al. 1990; Pereira 1993). As a response to this energetic stress, females evolved priority of access to food resources, leading eventually to unconditional female dominance, which characterizes most lemurs today (Jolly 1984). In order to maintain female dominance, females may have reduced male intrasexual competition by synchronizing their fertile periods and by choosing small males as mates, thus preventing the evolution of sexual dimorphism (Richard 1992).

Although this hypothesis is appealing, the basic premises it rests upon have to be questioned (reviewed in van Schaik and Kappeler 1996). The main assumption of unusually high energetic costs posed upon lemur females was not supported because postnatal growth rates are not higher (Kappeler 1996) and milk quantity and composition is not more costly in lemurs than in other strepsirrhines lacking female dominance (Tilden and Oftedal 1995). In addition the hypothesis focuses only on one lemur trait, i.e. female dominance.

### 2. Groups of multiple pairs hypothesis (van Schaik and Kappeler 1993)

Starting from the observation that within multi-male - multi-female lemur groups, pairs of individual males and females exist which are characterized by frequent affiliative and infrequent agonistic interactions (Kappeler 1993), it was postulated that bisexual lemur groups are comprised of multiple pairs (van Schaik and Kappeler 1993; see also Jolly 1998). This hypothesis rests on two premises: First, the risk of infanticide seems to be modulated by the mode of infant care, with females that carry their young facing a higher risk of infanticide than female who park



their infants (van Schaik and Kappeler 1997). Infant-carrying females are thus expected to permanently associated with at least one protector male. Second, predation risk will lead to group-living in diurnal species (e.g. van Schaik 1983), whereas nocturnal species forage alone because the advantage of group living with respect to predator avoidance is reduced during nocturnal activity (Terborgh and Janson 1986). In cathemeral lemurs these two factors should consequently lead to pair-living at night and the formation of groups comprising multiple pairs during diurnal activity.

The hypothesis proposes female estrous synchrony to be the mechanism maintaining the pair structure by lowering male monopolization potential. This in turn will lead to mating privileges between pair-partners, reduced male intrasexual competition, lack of sexual dimorphism in body and canine size and possibly the evolution of female dominance. A major weakness of the hypothesis is, however, that it does not explain the universality of these traits in all group-living lemurs, i.e. the diurnal species. Maybe even more importantly, several studies of the social organization and the mating system of cathemeral redfronted lemurs did not support the most basic prediction. Groups did not split up into pairs during nocturnal activity (Ostner and Kappeler 1999). In addition, while evidence of special relationships between individual males and females emerged from studies in captivity (Kappeler 1993; Pereira and McGlynn 1997), studies of wild populations did not support this prediction of the hypothesis (Overdorff 1998; Ostner and Kappeler 1999). Instead either functional one-male groups with a central, dominant male per group were found (Ostner and Kappeler 1999) or no clear structure of association and mating patterns could be detected (Overdorff 1998). The prevalence of groups structured around one particular male has now been confirmed for more groups and over a longer period of time in the Kirindy population, clearly showing that matings are skewed towards the dominant male (Chapter 2; Ostner unpubl. data). Recent genetic analyses revealed that paternity was also concentrated on the dominant male (Wimmer and Kappeler 2002). Together these findings contradict basic assumptions of the hypothesis of multiple pairs.

### 3. Evolutionary disequilibrium hypothesis (van Schaik and Kappeler 1996)

While the “groups of multiple pairs hypothesis” assumes that cathemerality reflects an old and stable adaptation, the “evolutionary disequilibrium hypothesis”

proposes that recent ecological changes on Madagascar, i.e. the extinction of large diurnal raptors (Goodman 1994a; b), caused a shift in some taxa from a nocturnal towards a more diurnal life-style (van Schaik and Kappeler 1996). Consequently, currently diurnal and cathemeral lemurs are in a transitional state from nocturnal infant-carrying species living in associated pairs (van Schaik and Kappeler 1993) to diurnal groups. The combination of unusual lemur traits reflects the still ongoing convergence with diurnal group-living anthropoid primates. In the light of the evolutionary disequilibrium, the discrepancy between anthropoid-like behavioral patterns (functional one-male groups, male dominance relations, paternity concentration) and morphological and demographic features characteristic for pair-living species (even sex ratio, lack of sexual dimorphism), which also have been confirmed in this study, is plausible. However, although these results of behavioral studies do not explicitly contradict the hypothesis, because behavioral traits may evolve faster than morphological features, the data do also not support it. Results in favour of the hypothesis would be evidence for special relationships, mating privileges between pair partners and genetic monogamy.

Unfortunately, it is not possible to test this non-adaptive hypothesis directly. Van Schaik and Kappeler (1996) support the hypothesis mainly by indirect evidence, showing for example that the visual system of diurnal lemurs is not different from the system of nocturnal lemurs, which indicates that diurnal lemurs are not adapted to day vision. However, a recent study correlating parameters of the visual system and activity pattern revealed that diurnal and nocturnal lemurs can be distinguished on the basis of morphological features related to vision and that diurnal lemurs are clearly specialized for photopic vision (Kay and Kirk 2000). To reach a final verdict more data on day/night contrasts in behavioral, ecological and morphological adaptations are needed.

#### 4. Energy frugality hypothesis (Wright 1999)

This hypothesis is a modulation and extension of the “energy conservation hypothesis”, resting also on the assumption that Madagascar poses a unique ecological challenge to its fauna (Wright 1999). According to this hypothesis, the combination of nine of the above mentioned unusual traits (only unusual group composition/even adult sex ratios are not addressed) constitute adaptations either to

conserve energy (as already proposed by the “energy conservation hypothesis”) or to maximize use of scarce resources. Traits proposed to conserve energy are for example low basal metabolic rate, abundance of pair-living and seasonal breeding, whereas a cathemeral life-style and female dominance are assumed to maximize the use of resources (Wright 1999). According to the hypothesis the combination of these traits evolved as a response to the unusually harsh and unpredictable environment of Madagascar.

This hypothesis helps to explain most proposed lemur idiosyncrasies, although it does not explicitly explain the lack of single-male groups and the even sex ratio. However, it proposes strong breeding seasonality and weaning synchrony as mechanisms to conserve energy and maximize resource exploitation. These factors may facilitate estrous synchrony, which will hamper the formation of single-male groups. Small female group sizes, in combination with regular eviction of females, indicate the existence of strong within-group feeding competition (van Schaik 1989; Sterck et al. 1997). Strong within-group feeding competition may be caused either by the proposed high energetic needs of lemur females (Wright 1999) or alternatively by the scarcity and/or unfavourable distribution of high quality food resources (van Schaik 1989). In order to evaluate the “energy frugality hypothesis”, additional data on nutritional needs and metabolic strategies are needed. In addition, further research is needed to decide whether the ecological setting of Madagascar is as special and unpredictable as proposed by the hypothesis. It seems that other habitats are exposed to similar pressures, but animals did not evolve the same suite of traits.

In conclusion, no single hypothesis seems to be sufficient to explain all unusual lemur traits. While the “groups of multiple pairs hypothesis” has to be rejected on the basis of recent behavioral and genetic analyses, the “energy conservation hypothesis” has been extended into a promising new version, i.e. the “energy frugality hypothesis”. The “evolutionary disequilibrium” also provides a comprehensive explanation for lemur idiosyncrasies, although recent morphological results weakened the hypothesis. The explanation derived from my study relating male life history and group composition provides an additional testable scenario for the evolution of unusual sex ratios in redfronted lemurs. Because the hypotheses are

not necessarily exclusive, further research should attempt to integrate the various aspects of the proposed explanations.

# SUMMARY

The number of males in animal groups is an essential determinant of male and female reproductive strategies. Females may benefit from living with several males, whereas males generally strive to monopolize a group of females. Due to male intrasexual competition, the sex ratio of groups of anthropoid primates is generally female-biased. Gregarious Malagasy lemurs deviate from theoretical expectations derived from sexual selection theory and from patterns found among anthropoids because they live in relatively small groups with an even or male-biased adult sex ratio and lack sexual dimorphism. The aim of this thesis was to investigate sex-specific reproductive strategies relating to the unusual group composition of redfronted lemurs (*Eulemur fulvus rufus*) by combining behavioral, demographic and endocrinological data.

In the first of a set of four studies I investigated the applicability of non-invasive endocrine measurements for monitoring ovarian function in wild redfronted lemur females in order to evaluate the degree of estrus synchrony. Further, I tested the prediction that males living in multi-male groups rely on indirect mechanisms of intrasexual competition, such as physiological suppression of testicular function. Several possible benefits gained from living with many males have been proposed and the hypothesis that additional males improve social thermoregulation was tested in the third study. Finally, I examined the proximate determinants of the unusual sex ratio within groups, the variation in the adult sex ratio as well as possible social benefits of the high number of males for both sexes. The study was conducted in Kirindy Forest, Madagascar, between April 1999 and July 2000. I recorded >3000 hours of focal animal data on social and sexual behavior of all adult members of five groups. Additionally, >2200 fecal samples of males and females were collected for subsequent hormone analysis using enzymeimmunoassay (EIA). Further, I analyzed demographic data from seven *Eulemur fulvus rufus* groups collected between 1996 and 2002.

The analyses of fecal estrogen and progesterone excretion in wild and captive females revealed that monitoring ovarian function is principally possible in redfronted lemurs, as demonstrated by the analysis of samples from captive females. Characterization of ovarian cycles in wild females, however, was not possible, because of a high day-to-day variability in excreted hormones. Nevertheless, the

study provided reliable information on gestation and cycle length as well as endocrine changes associated with gestation. Additionally, I established a method for prenatal sex determination using maternal fecal samples collected during late gestation. The excretion pattern of androgens in samples of males revealed no differences between dominant and subordinate males, indicating that dominant males did not suppress the endocrine function of subordinate rivals. High frequencies of matings in combination with large testes size suggest that male reproductive competition relies at least partly on sperm competition. Females did not benefit from the high number of males in their groups in terms of improved thermoregulation because surplus males did not participate frequently in huddling groups with females. Analysis of the demographic data revealed that birth and mortality rates were not sex-biased and that males migrated considerably more frequently than females, providing no proximate explanation for the unusual sex ratio. Females in this study may proximately regulate group composition by synchronizing their fertile periods, which were inferred indirectly from the temporal distribution of births within groups. Both males and females benefit from the high number of co-resident males because reduced male group size seemed to be the main predictor of take-over rate, and thus, infanticide risk.

The results of these studies suggest that certain life history traits (fast maturation, short inter-birth intervals) may ultimately determine the high number of males and the lack of single-male groups seen in redfronted lemurs. An accelerated male life history may facilitate joint group transfers and take-overs of male coalitions without a transitional time outside bisexual groups. Because males and females both benefit from a high number of males the conflict of interests between the sexes is considerably defused.

# ZUSAMMENFASSUNG

Die Anzahl adulter Männchen innerhalb einer Gruppe von Tieren stellt eine wesentliche Determinante männlicher und weiblicher Reproduktionsstrategien dar. Weibchen profitieren in der Regel davon, wenn mehrere Männchen in ihrer Gruppe leben, während Männchen bestrebt sein sollten, eine Gruppe von Weibchen zu monopolisieren. Aufgrund intrasexueller Konkurrenz unter Männchen ist das Geschlechterverhältnis in Gruppen anthropoider Primaten zugunsten der Weibchen verschoben. Gruppenlebende madegassische Lemuren weichen von den aus der Sexuellen Selektionstheorie hergeleiteten Erwartungen, sowie von den bei anthropoiden Primaten beobachteten Mustern ab. Sie leben in relativ kleinen Gruppen mit einem ausgeglichenen oder zugunsten der Männchen verschobenem Geschlechterverhältnis und zeigen keinen Sexualdimorphismus. Ziel dieser Studie war die Untersuchung geschlechtsspezifischer Reproduktionsstrategien im Zusammenhang mit der ungewöhnlichen Gruppenzusammensetzung von Rotstirnmakis (*Eulemur fulvus rufus*) durch eine Kombination von Verhaltensbeobachtungen mit endokrinologischen und demographischen Daten. In der ersten von vier Teilstudien untersuchte ich, ob die ovarielle Funktion mittels non-invasiver Hormonmessungen aufgezeichnet werden kann. Damit sollte der Grad an Östrussynchronisation analysiert werden. Anschließend überprüfte ich die Vorhersage, daß Männchen in Mehrmännchengruppen indirekt konkurrieren, indem sie z. B. die testikuläre Funktion ihrer Rivalen physiologisch unterdrücken. Es wurden mehrere mögliche Vorteile des Lebens mit mehreren Männchen vorgeschlagen und im dritten Teil dieser Arbeit wurde die Hypothese getestet, daß zusätzliche Männchen die soziale Thermoregulation verbessern. Schließlich untersuchte ich die proximalen Determinanten des ungewöhnlichen Geschlechterverhältnisses, die Variation im Adultgeschlechterverhältnis sowie mögliche soziale Vorteile der hohen Männchenzahl für beide Geschlechter. Die Untersuchung wurde zwischen April 1999 und Juli 2000 im Kirindy Wald in Madagaskar durchgeführt. Ich sammelte über 3000 Stunden Fokustierbeobachtungen zum Sozial- und Sexualverhalten aller adulter Tiere aus fünf Gruppen. Zusätzlich wurden über 2200 Kotproben von Männchen und Weibchen zur anschließenden Hormonanalyse mittels Enzymimmunoassays (EIA) gesammelt. Schließlich analysierte ich demographischen Daten, die von sieben Rotstirnmakigruppen zwischen 1996 und 2002 erhoben wurden. Die Analyse der

Östrogen und Gestagenexkretion von Weibchen aus dem Zoo zeigte, daß die Aufzeichnung ovarieller Funktion bei Rotstirnmakis prinzipiell möglich ist. Die Charakterisierung ovarieller Zyklen anhand der Proben aus dem Freiland war jedoch nicht möglich, da die Hormonkonzentration einer starken Tag-zu-Tag Schwankung unterworfen war. Nichtsdestoweniger lieferte die Studie zuverlässige Informationen zu Gestations- und Zykluslänge sowie zu endokrinologischen Veränderungen während der Schwangerschaft. Dadurch konnte ich eine Methode zur pränatalen Geschlechtsbestimmung durch die Analyse maternaler Kotproben entwickeln. Proben von dominante und subordinate Männchen unterschieden sich nicht in ihrer Androgenkonzentration, was darauf hinweist, daß dominante Männchen ihre Rivalen nicht physiologisch unterdrückten. Die Kombination aus häufigen Paarungen und großen Hoden könnte als Hinweis darauf gewertet werden, daß indirekte männliche Konkurrenz auf der Ebene der Spermien stattfindet. Weibchen profitierten nicht von der hohen Zahl an Männchen in Form verbesserter Thermoregulation, da die zusätzlichen Männchen die Ruhephasen nicht häufig im Körperkontakt mit Weibchen verbrachten. Die Analyse der demographischen Daten bot keine proximate Erklärung für das ungewöhnliche Geschlechterverhältnis. Die Geburts- und Mortalitätsrate war für beide Geschlechter gleich hoch und Männchen migrierten sehr viel häufiger als Weibchen. Durch Synchronisierung ihrer fertilen Phasen, die indirekt aus der Verteilung der Geburten berechnet wurden, beeinflussten Weibchen proximat die Gruppenzusammensetzung. Sowohl Männchen wie auch Weibchen profitierten von der großen Zahl Männchen in ihrer Gruppe, da eine geringe Männchenzahl der wesentliche Einflußfaktor für das Auftreten von Gruppenübernahmen, und folglich auch von Infantizid, war. Die Ergebnisse dieser Studien deuten an, daß bestimmte Lebenslaufparameter (schnelle Reifung, kurzes Intergeburtenintervall) ultimata die große Anzahl an Männchen und das Fehlen von Einmännchengruppen bei Rotstirnmakis bestimmen. Ein beschleunigter Lebenslauf bei Männchen könnte gemeinsame Migrationen und Gruppenübernahmen durch Koalitionen von Männchen ermöglichen, ohne daß Männchen die Kosten einer Übergangszeit außerhalb bisexueller Gruppen tragen müssen. Da beide Geschlechter Vorteile aus der großen Männchenzahl ziehen, wird der Interessenskonflikt zwischen den Geschlechtern im Hinblick auf die Gruppenzusammensetzung deutlich entschärft.



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# APPENDIX

Acronyms of males (numbers) and females (letters) of the seven study groups and corresponding names; in some publications only first three letters of names are used.

<b>Acronym</b>	<b>NAME</b>	<b>Acronym</b>	<b>Name</b>
1	Krk	48	Japan
2	Rhodos	49	Nicobar
3	Zypros	50	Anadaman
4	Bali	51	Bhutan
5	Komodo	52	Capri
6	Singapur	53	Alor
7	Isar	54	Obi
8	Neckar	55	Vincent
9	Main	56	St.Thomas
10	Alster	57	Montego
11	Rhein	58	Giglio
12	Lemnos	59	Kos
13	Borneo	60	Wisconsin
14	Lombok	61	Fidel
15	Sulawesi	AC	Corsica
16	Donau	AE	Elba
17	Ems	AS	Sicilia
18	Stromboli	ASa	Sardegna
19	Vulcano	AK	Kreta
20	Hongkong	BJ	Java
21	Puerto	BS	Sumatra
22	Tobago	BT	Tonga
23	Newcomer	BG	Guinea
24	Rico	BP	Papua
25	Pagai	BSi	Sipora
26	Barbados	BF	Flores
27	Haiti	BL	Lingga
28	Congo	BK	Kuta
29	Niger	BSb	Siberut
30	Sudan	DM	Mosel
31	Malawi	DS	Saar
32	Maroc	FC	Cuba
33	Gabon	FG	Grenada
34	Mali	FL	Lucia
35	Split	HA	Arizona
36	Timor	HN	Nevada
37	Key	IK	Kenia
38	West	IN	Namibia
39	Largo	IA	Angola
40	Schwarzkopf	JC	China
41	Splitear	JI	India
42	Rotbart	JB	Burma
43	H-drei	JK	Korea
44	Thai	JL	Lanka
45	Vietnam	JCa	Cambodia
46	Laos		
47	Nepal		