The hidden matrilineal structure of a solitary lemur: implications for primate social evolution

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Kin selection affects many aspects of social behaviour, especially in gregarious animals in which relatives are permanently associated. In most group-living primates with complex social behaviour, females are philopatric and organized into matrilines. Models of primate social evolution assume that females in solitary primates are also organized into matrilines. We examined the genetic structure and the mating system of a population of Coquerel's dwarf lemur (*Mirza coquereli*), a solitary primate from Madagascar, to test this assumption. Our genetic and behavioural analyses revealed that this population of solitary individuals is indeed structured into matrilines, even though this pattern was not predicted by behavioural data. Specifically, females sharing a mitochondrial DNA haplotype were significantly clustered in space and the average genetic and geographical distances among them were negatively correlated. Not all females were philopatric, but there is no evidence for the successful settlement of dispersing females. Although not all adult males dispersed from their natal range, they were not significantly clustered in space and all of them roamed widely in search of oestrous females. As a result, paternity was widely spread among males and mixed paternities existed, indicating that scramble competition polygyny is the mating system of this species. Our data therefore revealed facultative dispersal in both sexes with a strong bias towards female philopatry in this primitive primate. We further conclude that complex kinship structures also exist in non-gregarious species, where their consequences for social behaviour are not obvious.

Keywords: philopatry; dispersal; kinship; polygyny; primates; evolution

1. INTRODUCTION

Patterns of kinship are used to explain affiliative and cooperative behaviour within the framework of inclusive fitness (Hamilton 1963) and kin-selection theory (Maynard Smith 1964; Wilson 1975) in a wide variety of taxa, including mammals (e.g. Sherman 1977; Packer *et al.* 1991; Clutton-Brock *et al.* 2001). Affiliation and cooperation are also salient aspects of primate social behaviour (van Hooff & van Schaik 1992; Mitani *et al.* 2000) that are expected to be especially common among relatives (Gouzoules 1984; Moore 1992; Chapais 2001; Silk 2002). Studies of anthropoid primates with known genetic structures have demonstrated that cooperative behaviour is indeed affected by the degree of kinship in the predicted manner (Morin *et al.* 1994; Alberts 1999; Pope 2000*a*; Chapais *et al.* 2001; Silk 2002). However, recent genetic and behavioural studies of bonobos (*Pan paniscus*) and common chimpanzees (*Pan troglodytes*) have revealed that affiliation, cooperation and bonding also occur in the absence of close genetic ties (Goldberg & Wrangham 1997; Gerloff *et al.* 1999; Mitani *et al.* 2000; Vigilant *et al.* 2001), emphasizing the fact that investigations into the origins and consequences of particular kinship patterns remain central in the complete analyses of (primate) social structure and evolution (e.g. Perrin & Mazalov 2000).

Most primates and other mammals are characterized by female philopatry and male dispersal (Greenwood 1980; Pusey & Packer 1987; but see Moore 1984; Strier 1999), indicating that this character combination represents the ancestral condition. Facultative dispersal by both sexes is common in only a few primate taxa, including some atelines, colobines and gorillas (*Gorilla gorilla*) (Watts 1990; Pope 2000*b*; Steenbeek *et al.* 2000). In bonobos, chimpanzees and a few other species, males are the resident sex and females the dispersing sex (Struhsaker 1980; Strier 1999; Mitani *et al.* 2000). Because members of the resident sex are expected to become closely related over evolutionary times (Hoelzer *et al.* 1994; Altmann *et al.* 1997; de Ruiter & Geffen 1998; but see above), thereby generating brotherhoods or matrilines of closely related members of the same sex, understanding the evolution of sex-biased dispersal lies at the core of all ultimate analyses of social behaviour (Gandon 1999; Storz 1999; Perrin & Mazalov 2000).

It is generally assumed that female primates derive, on average, greater benefits from remaining in their natal area and that males benefit more from dispersing because of the nature of the respective benefits in relation to the sex-specific determinants of reproductive success (Wrangham 1980; Pusey & Packer 1987; Clutton-Brock 1989). It remains poorly understood, however, to what extent dispersal decisions and the resulting genetic structure are affected by demographic (Pope 1998), environmental (Richardson *et al.* 2002) and social (Dobson 1998) factors. Furthermore, only a few studies have combined
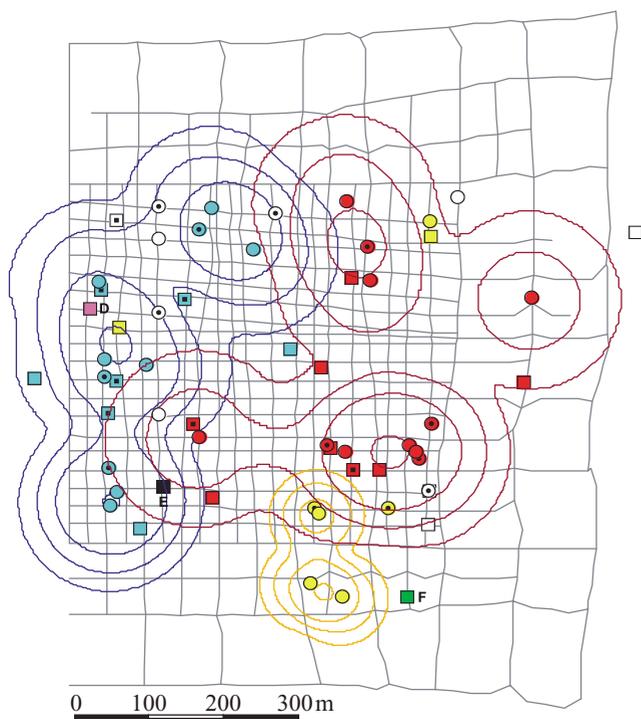
Figure 1. Spatial distribution of *M. coquereli* in Kirindy Forest. The centres of activity of all animals (circles, females; squares, males; undotted symbols, adults; dotted symbols, juveniles) co-residing in the study area in 1995 are shown within the trail system of the study area. The mtDNA haplotypes are indicated by colours (red, haplotype A; blue, haplotype B; yellow, haplotype C; open, haplotype not determined; letters, rare haplotypes). Males D and E were captured in 1994 and 1997, respectively, but are included to illustrate the male dispersal observed. The plots of the 95, 75, 50 and 5% Kernel densities contours (Silverman 1986) for adult females with common haplotypes are also depicted. One female of haplotype C was excluded from these calculations because she was captured only once north of the haplotype A cluster. The sample size for haplotype C was too small to determine the significance of its clustering.

detailed genetic and behavioural data to characterize genetic structures and dispersal strategies of solitary mammals (Waser & Jones 1983; van Staaden *et al.* 1994) and, to our knowledge, there are no studies on solitary primates (Müller & Thalmann 2000), so that the potential evolutionary pathways between solitary and gregarious social systems of primates remain poorly understood. In this paper, we present a characterization of the genetic structure of a solitary prosimian primate to provide an initial reference point for more comprehensive comparative studies of the evolution of primate dispersal and kinship structure. By taking this comparative perspective, we do not wish to imply that observed aspects of social organization are not the result of current prevailing selective pressures; rather, we want to emphasize that consideration of the phylogenetic history of a trait can also contribute to explanations of behaviour (Tinbergen 1963; Sherman 1988), for example by identifying possible or probable evolutionary transitions (Foley & Lee 1989; Kappeler 1999).

The existing hypotheses about evolutionary transitions among primate social systems assume that the formation of permanent, female-bonded groups consisting of one or several associated matrilines (Melnick 1987; Silk 2002) was facilitated among diurnal primates by several benefits, including those derived from the existence of matrilineal structures in their solitary ancestors (Charles-Dominique & Martin 1970; Martin 1972; Charles-Dominique 1978; Clark 1978; Wrangham 1980; van Schaik & van Hooff 1983; Foley & Lee 1989), whereas the origin of gregarious species with female or bisexual dispersal is explained with other sex-specific social benefits, such as improved mate defence or infanticide protection (Pusey & Packer 1987; Sterck & Korstjens 2000). Thus, daughters of solitary primate females are assumed to remain in their natal area and to settle and breed close to their mother. Reproductive rates permitting, female members of more than two generations may even become spatially clustered. This assumption also implies and predicts male-biased dispersal (Greenwood 1980). However, to date only circumstantial evidence for female philopatry and male-biased dispersal has been presented for solitary primates (Charles-Dominique 1977; Waser & Jones 1983; Clark 1985).

In fact, the diversity of social organizations and mating systems of the more than 60 species (Bearder 1999) of nocturnal prosimian primates (i.e. lemurs, lorises and tarsiers) has remained largely in the dark (Kappeler & van Schaik 2002). While some nocturnal prosimians are pair-living, most appear to live in more complex societies, characterized by predominantly solitary foraging at night and gregarious resting during the day (Charles-Dominique 1977; Müller & Thalmann 2000). The typical mating system of solitary prosimians is supposed to be spatial polygyny (Charles-Dominique 1977; Bearder 1987; Müller & Thalmann 2000), where an adult male monopolizes access to the ranges of several females, but other forms of polygyny have also been identified (Kappeler 1997a; Eberle & Kappeler 2002). The genetic structure of populations of solitary primates is unknown, however, even though crucial insights into primate social evolution could be gained from a detailed understanding of kinship patterns in these taxa (see above and Ross 2001).

To begin testing the assumptions about the genetic structure of solitary primates, we studied the social structure of a population of Coquerel's dwarf lemur (*Mirza coquereli*) in Kirindy Forest, Western Madagascar (Kappeler 1997a). Since 1993, more than 100 individually marked animals of this 300 g nocturnal cheirogaleid have been regularly captured and observed within a 60 ha study area. The field studies revealed that adult individuals in this population rarely share nests and typically forage alone. Females, which can begin reproducing within their first year of life, occupied home ranges of about 4 ha that remained stable in size and location throughout the year. The home-range overlap among neighbouring females was highly variable, ranging from 0 to 99% with one to eight neighbours, and active defence was never observed. The proportion of females recaptured after 3 years was three times higher than for males, indicating higher rates of male mortality and/or dispersal, but unmarked adult males and females continued to be captured several years into the study, indicating that both sexes may leave their natal ranges. During the brief annual mating season, males more than quadrupled their ranges

Table 1. Haplotype diversity and sample size by sex and age of the 1995 cohort.

(f, female; m, male; ?, unknown sex; ad, >1 year; juv, <1 year; A, B, C, F, number of (individuals) with different mtDNA haplotypes; r = number of (individuals) with nuclear data available; N = sample size.)

sex	age	N	A	r	B	r	C	r	F	r	N (no mt)	N (no r)	N (mt)	N (r)
f	ad	21	7	7	7	7	4	4	0	0	3	1	18	19
m	ad	14	6	6	3	3	2	2	1	1	2	2	12	14
f	juv	12	4	4	3	3	2	2	0	0	3	1	9	10
m	juv	8	2	2	5	5	0	0	0	0	1	1	7	8
?	juv	1	0	0	0	0	0	0	0	0	1	0	0	0
total		56	19	19	18	18	8	8	1	1	10	5	46	51

and roamed widely in search of oestrous females. Relatively large testes indicate that sperm competition is the main mechanism of male reproductive competition (Kappeler 1997*b*).

Together, these field observations indicated that females may be the more philopatric sex and that scramble competition polygyny may be the mating system of this population. We tested both of these predictions with genetic data, but we stress that the available behavioural data provided no basis for predicting a particular genetic structure of this population of solitary individuals.

2. MATERIAL AND METHODS

The study site and field methods are described in detail in Kappeler (1997*a*). For the present analyses, data were available from 104 animals captured and observed between 1993 and 1998. We obtained tissue samples for later DNA extraction and analyses from 68 of these individuals. The statistical analyses presented in § 2a focus on 56 individuals that were co-resident in the study area in 1995, including a cohort of 16 infants born that year, because meaningful statistical comparisons are not possible with data from subsequent years. 1995 was the year with the highest data density; the data obtained from other years are in general agreement with our conclusions (see electronic Appendix A available on The Royal Society's Publications Web site).

(a) *Genetic analyses*

The DNA for genetic analyses was extracted from small skin biopsies obtained from a total of 68 individuals. For 46 of the 56 co-resident individuals in 1995, we were able to determine the mitochondrial DNA (mtDNA) haplotype, and for 51 of them we were able to estimate pairwise relatedness based on the analysis of nuclear DNA. In addition, we included nine individuals from a population *ca.* 4 km away to assess the genetic similarity between the two populations. The distribution of the mtDNA haplotypes across the age–sex classes of our primary sample are shown in table 1 to illustrate the variable sample sizes in the analyses presented herein.

Each piece of tissue was ground and incubated overnight at 37 °C in 200 µl of lysis buffer containing 100 mM Tris–HCl (pH 8), 80 mM ethylene diamine tetra-acetic acid (pH 8), 0.5% sodium dodecyl sulphate and 10 µl proteinase K (15 mg ml^{-1}) and was then subjected to a standard phenol–chloroform extraction (Sambrook *et al.* 1989). A 530 bp fragment of the mitochondrial D-loop was amplified via PCR using the mammalian control region primers L15997 5′-CACCATTAGCACC CAAAGCT-3′ located in the tRNA gene and H16498 5′-CCTGAAGTAGGAACCAGATG-3′ (Gerloff *et al.* 1999). In a 25 µl reaction, *ca.* 5–10 ng of template, 1 µM of each primer,

0.2 mM of both dNTP (2′-deoxynucleoside 5′ triphosphate) and 0.5 U AmpliTaq (thermostable Taq DNA polymerase, derived from *Thermus aquaticus*; Perkin–Elmer, Princeton, NJ, USA) with PCR buffer were used. The cycling conditions were as follows: an initial denaturing step of 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. Amplification was always carried out with a negative control to test for contamination and reactions checked on an agarose gel.

Prior to sequencing, the amplification products were purified using Ultrafree-MC centrifugal filter units (regenerated cellulose, 30 000 nominal molecular weight limit). A total of 30–90 ng of the purified product were used for sequencing both strands of the fragment directly with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin–Elmer) and the previously mentioned primates. The sequence reaction products were electrophoresed on 5% acrylamide gels on an automated sequencer ABI 377 XL automated sequencer (Perkin–Elmer). The raw data were analysed with SEQUENCE ANALYSIS 3.0 (Perkin–Elmer) and edited by hand in SEQUENCE NAVIGATOR 1.0.1 (Perkin–Elmer). The programs CLUSTAL V (Sambrook *et al.* 1989) and MACCLADE (Maddison & Maddison 1992) were used to align the sequences. Phylogenetic analysis of the haplotypes was conducted with the program PAUP 4.0 (D. L. Swofford—distributed by Sinauer, Sunderland, MA).

The PCR conditions for the five microsatellites used were as follows: 10 ng DNA, 200 µM of each dNTP, 1 µM of each primer (one of each primer pair was end-labelled with a fluorescent tag) and 0.5 U AmpliTaq with supplied standard buffer and bovine serum albumin (1 mg ml^{-1}). The cycling profiles were: initial denaturing step at 94 °C for 3 min, followed by 25 cycles (for loci Mm3 and AMCD1.3) and 30 cycles (for loci McD, Mm34 and McE) of denaturing at 94 °C for 1 min, annealing at 48 °C (Mm3 and AMCD 1.3) or 50 °C (McD, Mm34, McE) for 1 min, extension at 72 °C for 1 min; and a final extension step of 72 °C for 5 min. The amplification products were separated on a 4.5% acrylamid gel and run with an internal size standard in each lane (Rox 350; Perkin–Elmer) on an ABI 377 XL. The relatedness among the individuals (R) was estimated from the allele frequencies at the five microsatellite loci, using RELATEDNESS 5.0 (Queller & Goodnight 1989). The results were evaluated with the help of the program CERVUS (Marshall *et al.* 1998). Due to the fact that infants are initially parked in nests and can only be captured once they begin travelling independently, both their potential mothers and fathers were initially unknown. We therefore used age, home-range data and genetic compatibility in an initial screening step to reduce the number of potential parents. Information on the primers and loci are available on request from the authors.

(b) *Analyses of the spatial data*

Female *Mirza* can begin reproducing within their first year of life (see also Stanger *et al.* 1995), so that the juvenile period lasts only a few months and the distinction between juveniles and adults becomes problematic for some analytical purposes. Nevertheless, we considered animals in their first year of life as juveniles and analysed spatial data from adults separately, sample size permitting.

We examined the spatial distribution of individuals with different mtDNA haplotypes with three independent methods. All analyses are based on coordinates of the centres of activity, which were calculated on the basis of an average of 10 captures per individual, in addition to the extensive radio-tracking of some individuals. First, we calculated the Kernel probability plots for adult females with common haplotypes with the Animal Movement (Hooge & Eichenlaub 1997) extension for ArcView. Second, we conducted nearest-neighbour analyses for all females and for adults separately (Clark & Evans 1954), also using the Animal Movement (Hooge & Eichenlaub 1997) extension for ArcView. The expected values for all nearest-neighbour analyses are based on an area of 62.55 ha and the one-tailed alpha-levels were set at 0.05 because we tested for clumpiness. Third, we compared the mean distances among the centres of activity of adult male and female dyads with identical haplotypes with distributions of mean distances among same-sexed dyads with different haplotypes, using a randomization test (Manly 1997) with 10 000 permutations and an adjusted alpha-level of 0.01 because the same dyads were used repeatedly in different tests.

To examine the long-term effects of sex differences in dispersal, we compared the distances between the annual centres of activity for a subset of individuals for which the spatial data from at least two subsequent years were available. To this end, we compared the means and variances of inter-annual distances between age and sex classes using *t*- and *F*-tests. Finally, we performed a Mantel test (Manly 1997) to examine the correlations between the relatedness values and geographical distances between the centres of activity for all pairwise combinations of males and females. We compared the calculated Mantel's *Z* statistics with distributions of *Z* generated by randomizing the geographical distance matrix 10 000 times.

3. RESULTS

The frequency distribution of the mtDNA haplotypes found within this population was highly skewed; three of them (A, B, C) were very common, whereas the remaining ones belonged to single adult males, who had apparently migrated into this population (table 1). The spatial distribution of haplotypes was highly structured, with animals that shared one of the common haplotypes forming nearly exclusive distinct clusters (figure 1). In three cases where one of the three common haplotypes was found within a cluster of individuals with another common haplotype, the respective animals were adult males, who apparently dispersed several hundred metres (i.e. several home-range diameters) away from their natal range. Only one female (haplotype C) was found a few hundred metres away from the other members of her haplotype. However, she was only captured once at the age of nine months and has not been recaptured since.

Nearest-neighbour analyses revealed a significant tendency towards clumping for activity centres of all females
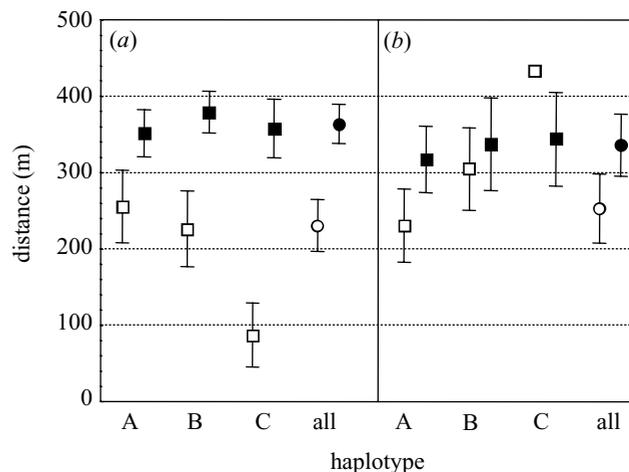
Figure 2. The comparison of the mean distances between the activity centres of (*a*) adult females and (*b*) males with the same or different haplotypes. Open symbols, the mean distance (± 95% confidence intervals) between activity centres of individuals sharing the same mtDNA haplotype; black symbols, the distance between the activity centres of individuals with different mtDNA haplotypes; squares, haplotypes A, B, C; circles, all haplotypes. Females with identical haplotypes were significantly closer to each other than to females with another haplotype (randomization test: A, $p < 0.01$; B, $p < 0.001$; C, $p < 0.001$; all, $p < 0.001$), whereas for males no such difference existed (all n.s.).

of haplotypes A ($R = 0.52$; $n = 11$, $z = -3.08$, $p < 0.01$), B ($R = 0.35$; $n = 10$, $z = -3.95$, $p < 0.001$) and C ($R = 0.23$; $n = 5$; $z = -3.28$, $p < 0.001$). This pattern was confirmed for adult females alone (haplotype A—$R = 0.72$, $n = 7$, $z = -1.42$, $p < 0.1$; haplotype B—$R = 0.40$, $n = 7$, $z = -3.02$, $p < 0.01$; haplotype C—$R = 0.28$, $n = 3$; $z = -2.40$, $p < 0.01$), but note that analyses with $N < 8$ must be treated with caution. The males of the three common mtDNA haplotypes were always much less clumped than the corresponding females and no significant clustering of males was detected.

The comparison of the mean distances between the centres of activity of adult female dyads with identical haplotypes to distributions of mean distances to other conspecifics with different haplotypes revealed that the mean distances between adult females sharing a haplotype were significantly shorter than the average distances to individuals with another haplotype, whereas for males, no such difference existed (figure 2). Thus, adult females sharing a mtDNA haplotype were significantly clustered in space. Furthermore, the mean distances between centres of activity in subsequent years did not differ significantly between the sexes, but males exhibited a significantly larger variance (figure 3). Between subsequent years, young females shifted their centre of activity significantly less than females over 2 years of age ($t = -2.12$, d.f. 22, $p < 0.05$), whereas the average mobility did not differ between young and older males ($t = -0.31$, d.f. 30, n.s.).

Relatedness at nuclear loci was negatively correlated with geographical distance in females, but not in males ($Z_F = -1543.7$, $p = 0.0087$, $R = -0.15$, $n = 27$; $Z_M = -1519.1$, $p = 0.1057$, $R = -0.09$, $n = 20$). Between the sexes, genetic and geographical distance were also negatively correlated ($Z_{F-M} = -23711.4$, $p = 0.0088$, $R =$

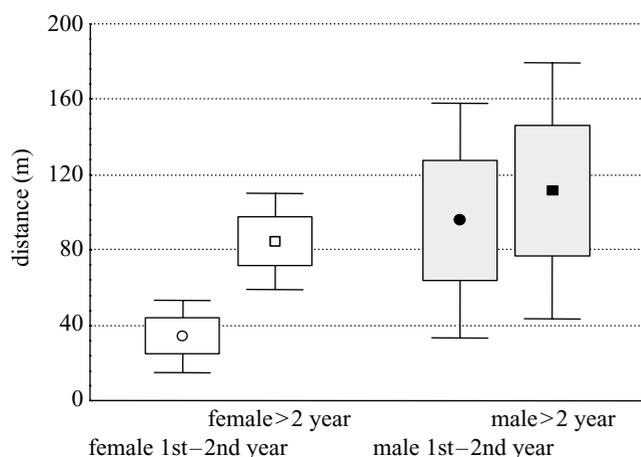Figure 3. The mean distances between the centres of activity of individual females (open) and males (grey) across subsequent years (mean ± s.e. (box) ± 95% confidence intervals (bars)). The mean distance does not differ between the sexes (adults: $t = -0.70$, d.f. = 36, n.s.; juveniles: $t = -1.33$, d.f. = 16, n.s.), but males have a significantly higher variance (adults: $F = 7.7$, $p < 0.0001$; juveniles: $F = 21.08$, $p < 0.01$).

-0.17, $n = 47$). For adults of both sexes alone, a similar pattern emerged ($Z_F = -2792.4$, $p = 0.0073$, $R = -0.22$, $n = 18$; $Z_M = -1985.9$, $p = 0.6941$, $R = -0.06$, $n = 13$).

To assess the microgeographical gene flow within the population, we also determined the mtDNA haplotypes from nine individuals caught in an area *ca.* 4 km away from the main study area. Only one adult male from this population shared haplotype A with individuals from our main study area. The remaining five females and three males belonged to five new haplotypes. This indicates that there must be an occasional transfer of haplotypes into other areas, but that these areas are also likely to consist of separately established clusters. The phylogenetic analysis of all haplotypes, which differed by a minimum of five and a maximum of 21 substitutions, revealed no evidence for long-term geographical differentiation at this scale, however (figure 4).

In an initial characterization of this species' mating system, we could exclude all but one adult female and male as putative parents for nine out of the 16 juveniles from the 1995 birth cohort, based on variability at five microsatellite loci. For an additional four juveniles, we could identify a putative mother but no compatible father. For one juvenile, maternity could be restricted to two closely related females, and for the remaining two juveniles all known adult females could be excluded as putative mothers. As a result, a total of nine females were identified as mothers of 13 juveniles, five of which had singletons and four of which had twins. For four of the 13 juveniles with identified mothers, all known resident males could be excluded as potential fathers, indicating that they were fathered by males who only briefly entered the study area during the mating season. A total of five adult males were responsible for the remaining nine paternities. Two males each could not be excluded as fathers of one or two infants, respectively, and one male was assumed to have fathered three juveniles. Two of the four pairs of twins had different fathers. This is, to our knowledge, the first
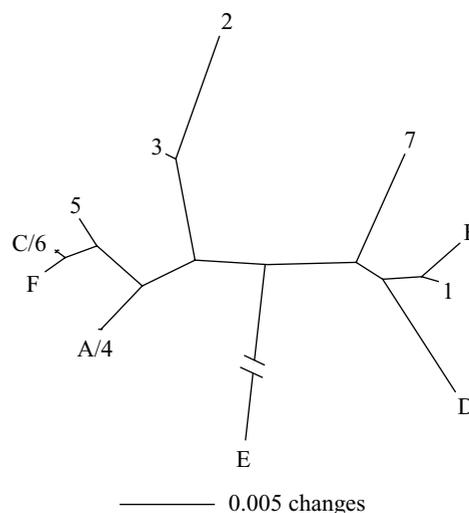
Figure 4. Unrooted phylogram of mitochondrial haplotypes from the study area (letters) and an area *ca.* 4 km away (numbers). There is no obvious relationship between position in the phylogram and geographical origin, indicating that there is a long-term mixing of haplotypes through occasional female migration, at least in this area. Note that haplotypes D and E come from two adult males that had entered our study area in 1994 and 1997, respectively. They have not been found in subsequent years. Haplotype F comes from a male that was found in our study area as a juvenile in 1993 and who stayed in the area until at least 1995. Only half of the length of the branch for haplotype E is shown.

demonstration of mixed paternities in a non-human primate.

4. DISCUSSION

The study revealed that solitary Coquerel's dwarf lemur females exhibit a hidden genetic structure at the population level that can be characterized as matrilineal clusters. It is intriguing that the existence of these clusters is not evident from behavioural interactions. We stress that the area was intensively studied for many years, but that the behavioural data alone provided no evidence for forms of female bonding, such as nest-sharing, or cooperation (Kappeler 1997*a*). Moreover, the habitat in this area is completely homogeneous and the borders between clusters do not correspond to any visible borders in the forest.

(a) *Female matrilineal clusters*

How do matrilineal clusters evolve and which mechanisms are employed to maintain them? Due to the fact that the existence of such clusters was not expected, we can examine these questions only indirectly with various *post hoc* analyses. To address the first question, we compared the results from the 1995 co-residents with those from the two previous years. Although fewer data are available from these years, the general distribution pattern is comparable with that presented in figure 1 (see electronic Appendix A).

Female philopatry is obviously instrumental in generating matrilineal clusters. Juvenile females were shown to shift their centres of activity little between their first and second years, whereas juvenile males tended to move, on
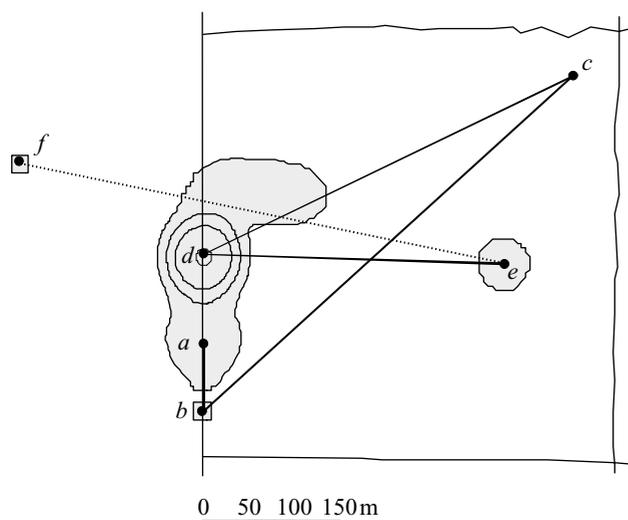
Figure 5. Unusual movements by female no. 44. This juvenile was radio-tracked in October 1995 when she was about nine months old, at about one month before her first breeding season. The resulting range is indicated by the 95, 75, 50 and 5% Kernel plots. The numbers indicate the sleeping sites between October and December, mostly outside her natal range. *a*, 11 October; *b*, 12 and 13 October; *c*, 14 October; *d*, 15 October and 30 November; *e*, 1, 2 and 3 December; *f*, 27 and 28 December. This female has not been recaptured or re-sighted since 28 December. Distances: *b–c*, 547 m; *c–d*, 447 m; *d–e*, 327 m; and *e–f*, 538 m. This case observation demonstrates that juvenile females can and do transfer distances corresponding to several home-range diameters, even within one day (solid lines).

average, much further. Thus, juvenile females were likely to settle near their mothers. However, because *M. coquereli* reproduce for less than five years and have up to five off-spring surviving to the age of first reproduction during this time (P. M. Kappeler, unpublished data), the matrilineal clusters are not simply composed of mother–daughter pairs. Instead, they must consist of females with a common ancestry going back for at least two generations. Furthermore, statistical analyses restricted to adult females also confirmed spatial clustering according to haplotype, indicating that it is not simply a passive result of daughters of the same generation settling near their mothers.

While female philopatry is involved in generating matrilineal clusters, it is apparently nevertheless facultative in this phylogenetically primitive primate. Two observations indicated that females also leave their natal area. One female with haplotype C was found only once in the haplotype A area and then disappeared. Another female with haplotype B was observed in the haplotype B area as a juvenile, but shortly before her first breeding season, at the age of about nine months, she moved over large distances well outside her normal range within a few days. Apparently, she could not establish herself in any of the locations that she visited and eventually disappeared (figure 5). Unfortunately, behavioural mechanisms determining the success of female dispersal remain elusive. However, while the exact fates of these two females remain unknown, their case studies nevertheless indicate the possibility that dispersing females with a different haplotype can only settle successfully in areas not occu-

pied by members of another matriline. In this case, one expects the overall female relatedness at nuclear loci to be negatively correlated with geographical distance, a pattern confirmed by our analyses.

Why female *Mirza* may benefit from philopatry is not apparent. If we assume that dispersal and philopatry are facultative options for females, the majority appear to have chosen philopatry. Improved foraging efficiency as a result of familiarity with an area has been proposed as the main benefit of female philopatry in primates (Pusey & Packer 1987), but *Mirza* is omnivorous with a large proportion of animal matter in its diet whose distribution is difficult to predict. Moreover, potential benefits from cooperative range defence with co-resident kin (Wrangham 1980) also do not accrue in this solitary species. Perhaps the knowledge of safe sleeping places is an important benefit of philopatry in such a small arboreal mammal. Future studies should focus specifically on the potential benefits of philopatry and potential intraspecific variability in dispersal by examining the breeding success of philopatric and dispersing females to compare the determinants and payoffs of these two strategies (e.g. Pope 2000b).

(b) *Male dispersal*

Philopatry and dispersal also appear to be facultative options for *Mirza* males. However, between the first and second years of life, young males shift their centre of activity on average more than twice as far as females of the same age and continue to shift it by a similar distance in all subsequent years. As indicated by the large variance in the mean distance between the annual centres of activity, some males move over relatively large distances—those leaving the study area are obviously not included in these analyses—whereas others are more sedentary. For example, one male (individual no. 76) was born in haplotype A area in 1995 and stayed there until at least 1998. Because we also found three adult males with new haplotypes in the area, we know that at least some were actively and successfully dispersing over much larger distances. However, only one of these three males remained in our study area for more than one year, indicating that males either have problems in establishing themselves outside their natal range, or that secondary dispersal is common. This question, as well as the possibility of population variation in dispersal indicated by the limited data from the second, distant population, can only be addressed in future studies with data from an even larger study area.

The proportion of males that disperse or, more generally, how variable rates of philopatry and dispersal are in both sexes (e.g. Woodroffe *et al.* 1993; Richardson *et al.* 2002) also needs to be determined in more extensive long-term studies. The suggested mating system of this species, in which roaming males can be expected to encounter more potential mates, may favour male dispersal. This benefit may also have provided an important evolutionary advantage (see Pusey & Packer 1987), but it raises poorly defined questions about the evolutionary dynamics and interactions among the social organization, mating system and genetic structure (Kappeler & van Schaik 2002). Thus, most males appear to leave their natal area early in life, possibly disperse over relatively large distances and perhaps shift their centres of activity on a frequent basis, but closer study is severely hampered by the logistical

problems associated with locating and following animals in this dense forest outside a trail system.

(c) Comparative aspects of genetic structure

The spatial clustering of mothers and offspring or among other close kin, which may affect aspects of female fitness (Lambin & Krebs 1993) and behaviour (Kawata 1987) has been demonstrated in a few other solitary mammals (Avise *et al.* 1979; van Staaden *et al.* 1994; Prodohl *et al.* 1998). However, the clustering of females according to haplotype within populations has, to our knowledge, only been documented in grey-sided voles (*Clethrionomys rufocanus*; Ishibashi *et al.* 1997). By contrast to *M. coquereli*, however, only members of some vole haplotypes exhibited clustering and, more importantly, grey-sided vole females occupy mutually exclusive home ranges. Thus, their female clusters presumably result from a combination of female philopatry, over-winter nest-sharing among related females and female territoriality, which constrains the options for the successful long-range dispersal of young females, whereas matrilineal clusters in *M. coquereli* exist in the absence of female nest-sharing and territoriality. Furthermore, parallel studies building on the present one (Radespiel *et al.* 2001; Wimmer *et al.* 2002) indicated similar female clustering and male dispersal in closely related grey mouse lemurs (*Microcebus murinus*), a species in which several related females also form stable sleeping groups, however.

The spatial clustering of solitary, matrilineally related females (as in *Mirza*) may therefore represent the evolutionarily most primitive situation, which may facilitate the establishment of more differentiated relationships (e.g. stable female sleeping groups in *Microcebus*). Upon becoming diurnal, such an organization into matrilines consisting of several related females and their offspring could facilitate the formation of permanent female-bonded groups in response to various other selective pressures. How groups with bisexual or female dispersal evolved needs to be explained based on comparisons with species with female philopatry (e.g. Pope 2000*b*).

(d) Mating system

Previous behavioural and morphological evidence had already indicated that scramble competition polygyny is the predominant mating system for *M. coquereli* (Kappeler 1997*a*,*b*). The paternity analyses presented in this paper have confirmed three predictions about this mating system with genetic data. First, litters with mixed paternity do exist. Second, individual males do not monopolize access to females in a particular area because infants sired by a particular male were not spatially clustered (data not shown). Finally, the small available sample indicates that variance in male reproductive success is not large. This form of polygyny, which is rare among mammals (Schwagmeyer 1988), is expected to occur when females are widely distributed in space and when their receptive periods are synchronized (Emlen & Oring 1977). However, a recent study of sympatric grey mouse lemurs revealed that males also roam widely in search of receptive females when their spatio-temporal distribution is such that they are in principle defendable (Eberle & Kappeler 2002). Monitoring the reproductive status of solitary females simultaneously at a population level in a small,

nocturnal arboreal primate poses the main obstacle in the future testing of this prediction in *Mirza*, however.

(e) Conclusions

Four main conclusions emerge from this study.

(i) Our results provide, to our knowledge, the first genetic support for a central assumption of the current theory of primate social evolution, as they show that a population of solitary primates possesses a matrilineal genetic structure.

(ii) We have demonstrated that complex kinship structures do also exist in non-gregarious primates.

(iii) This elaborated kinship structure exists in the absence of both obvious benefits and consequences for social behaviour.

(iv) These phylogenetically primitive primates exhibit a pronounced tendency towards female philopatry and male dispersal, but both dispersing females and non-dispersing males are not uncommon, indicating that sex-specific costs and benefits of philopatry and dispersal are not as canalized as in most gregarious anthropoid primates. Comparative genetic studies of a much wider variety of solitary primates are now required to evaluate the generality of the present results and conclusions.

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