

# Mating system, philopatry and patterns of kinship in the cooperatively breeding subdesert mesite *Monias benschi*

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## Abstract

In the first molecular study of a member of the threatened avian family, Mesitornithidae, we used nine polymorphic microsatellite loci to elucidate parentage, patterns of within-group kinship and occurrence of extra-group paternity in the subdesert mesite *Monias benschi*, of southwest Madagascar. We found this cooperatively breeding species to have a very fluid mating system. There was evidence of genetic monogamy and polygynandry: of the nine groups with multiple offspring, six contained one breeding pair with unrelated helpers and three contained multiple male and female breeders with related helpers. Although patterns of within-group kinship varied, there was a strong positive relationship between group size and relatedness, suggesting that groups form by natal philopatry. There was also a strong positive correlation between within-sex and between-sex relatedness, indicating that unlike most cooperatively breeding birds, philopatry involved both sexes. In contrast to predictions of kin selection and reproductive skew models, all monogamous groups contained unrelated individuals, while two of the three polygynandrous groups were families. Moreover, although between-group variation in seasonal reproductive success was related to within-group female relatedness, relatedness among males and between the sexes had no bearing on a group's reproductive output. While kin selection may underlie helping behaviour in females, factors such as direct long-term fitness benefits of group living probably determine helping in males. Of the 14 offspring produced by fully sampled groups, at least two were sired by males from neighbouring groups: one by a breeding male and one by a nonbreeding male, suggesting that males may augment their reproductive success through extra-group paternity.

**Keywords:** cooperative breeding, kinship, mating system, microsatellites, *Monias benschi*, subdesert mesite

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## Introduction

The evolution of cooperative breeding is widely viewed as a two-stage process: the decision to delay dispersal and the decision to help (Cockburn 1998; Nicholls *et al.* 2000). Although delayed dispersal does not invariably lead to helping, in most cooperative breeders it is a necessary prerequisite (Hatchwell & Komdeur 2000). The decision to stay is thought to be mediated by a combination of ecological constraints, life history factors and benefits of philopatry (reviewed in Pen & Weissing 2000). Conversely,

the decision to help is usually explained in terms of inclusive fitness benefits (Clutton-Brock 2002). For example, in family groups (i.e. the majority of cooperative breeders) subordinates either obtain indirect benefits by helping to rear kin, or they improve their chances of becoming breeders by remaining on the natal territory and helping (e.g. Emlen 1997). Conversely, in coalitions of unrelated individuals, help is only given by individuals that perceive they have direct paternity in the brood (Davies 2000). In both cases, subordinates may gain reproductive success directly (through descendent kin) or indirectly (through nondescendant kin). Subordinates obtain direct success through shared parentage within a group, extra-group fertilizations, or both (e.g. Davies 1992; Mulder *et al.* 1994). Indirect success

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may be gained through helping related breeders and thereby increasing their productivity or by increasing the survival of related parents (e.g. Emlen 1991).

The degree to which different group members contribute to reproduction in cooperative societies is influenced by patterns of dominance and relatedness within a group. Depending on the size and composition of the group, reproduction may either be highly skewed, with a single 'despotic' individual of each sex monopolizing reproduction, or more 'egalitarian', with many individuals sharing in reproduction. One series of 'reproductive skew' models proposes that subordinate reproduction is under dominant control, but that subordinates are occasionally allowed to reproduce as a concession in return for their help. Alternatively, the degree to which group members contribute to reproduction is partitioned may depend on the degree to which dominant individuals are able to control the reproduction of subordinates ('limited control' models).

We studied the influence of group composition, individual relatedness and reproductive skew on cooperative behaviour in the subdesert mesite *Monias benschi*, a group-territorial, terrestrial bird (Seddon *et al.* 2003) endemic to Madagascar and classified as Vulnerable to extinction according to the IUCN Red List criteria (BirdLife International 2000). Significant variability in group demography and patterns of dispersal suggest a high degree of social complexity in this species (Seddon *et al.* 2003). However, the evolutionary causes of this variability are unknown because the species' elusive nature and dense forested habitat make the collection of data on reproductive behaviour extremely difficult.

In other species with similarly intractable life histories, molecular genetic analyses have provided valuable insight into mating systems, relatedness, patterns of dispersal and levels of extra-group paternity (e.g. Koenig *et al.* 1996). Here we report on the first application of such molecular genetic analyses to any member of the gruiform family Mesitornithidae. With the ultimate aim of testing ideas about the importance of kinship in the evolution of cooperative breeding in birds, we investigate patterns of relatedness within social groups and relate these patterns to helping behaviour, reproductive success and reproductive skew.

## Materials and methods

### *Study species*

The subdesert mesite is an insectivorous gruiform bird belonging to a monotypic genus in the family Mesitornithidae that it shares with two other species, the white-breasted mesite *Mesitornis variegata* and the brown mesite *Mesitornis unicolor* (Evans *et al.* 1996). Although anatomically adapted to flight (Lowe 1924), all three species are mainly terrestrial and only fly to reach elevated roost sites or to avoid predators. While *Mesitornis* spp. are sexually

monomorphic, the subdesert mesite is dichromatic: the breast and throat of the female is rufous; that of the male is white with black crescents (Evans *et al.* 1996). Subdesert mesites breed in groups consisting of two to nine birds, each group containing an average of three adult males and two adult females. All adult males and at least one adult female contribute to incubation, parental care, and the defence of large (c. 12 ha), permanent, multipurpose territories. Groups produce one or two clutches of 1–2 eggs per year and hatchlings are precocial (Seddon *et al.* 2003). Social organization is very fluid: a preliminary genetic analyses using one polymorphic locus revealed that while some groups comprise related individuals, others contain coalitions of unrelated birds (Seddon 2001). Both sexes are essentially philopatric, but dispersal (or eviction) may be female biased (Seddon *et al.* 2003).

### *Study population*

The species is restricted to a 3700-km<sup>2</sup> area of semiarid coastal woodland and scrub in southwest Madagascar (Seddon *et al.* 2000), where its total population has been estimated at 115 000 individuals (Tobias & Seddon 2002). We studied a total of 23 unique groups during three field seasons (September–January 1997–2000; see Appendix I) at two sites in their natural range, separated by 6 km of contiguous vegetation: PK32 (23°04'57"S, 43°37'15"E; 200 ha) and Mangily (23°07'09"S, 43°37'30"E; 120 ha). The habitat at both sites consisted of a dense, xerophytic flora dominated by succulent and spiny plants, most notably members of the endemic family Didiereaceae (e.g. *Didierea madagascariensis*), woody euphorbias (e.g. *Euphorbia stenoclata*) and baobabs (e.g. *Adansonia fony*); see Seddon *et al.* (2000) for a description.

### *Behavioural observations*

We caught birds in mist-nets, attached individual combinations of coloured plastic leg-rings, and took 0.2 mL blood samples. We counted the number of individuals of each sex in each of the study groups at monthly intervals. A group was defined as a cohesive collection of individuals that shared a common territory, foraged together and cooperated over territory defence and the care of young. Groups were located by active systematic searches, chance encounters along forest trails, listening for distinctive vocalizations, finding and following characteristic tracks made in the sand and using radio transmitters (Seddon *et al.* 2003). Every few days groups were followed semi-continuously at a distance of 15–30 m, from dawn (c. 05:00 h) to 11:00 h and from 16:00 h to dusk (c. 19:00 h). There were too few behavioural observations to accurately quantify dominance interactions within groups. However, a male and female were considered the dominant pair in the

group if they consistently lead aggressive interactions against simulated territorial intrusions (Seddon 2001).

Because of seasonal changes in group composition, data were only available for a 5-month study period per group. Moreover, because mesites were studied for only three years, we were unable to measure individual fitness by estimating lifetime reproductive success. Instead, we use two estimates of seasonal reproductive output: (i) the total number of chicks that fledged, and (ii) the number of young that survived to at least three months of age (i.e. developed adult plumage; Seddon 2001). The capture and blood sampling of mesites was conducted under licence from the Ministry of Water and Forests (Antananarivo, Madagascar).

### *Molecular analysis*

Blood samples were taken from the brachial vein of all juvenile and adult birds and from the tarsal vein of all chicks and were stored in 1.0 mL of 100% ethanol. DNA was extracted using a salting-out procedure (Bruford *et al.* 1992) and was then enriched for GT repeat-containing fragments in polymerase chain reactions (PCR), following Gardner *et al.* (1999) with modifications as detailed in Adcock & Mulder (2002). Methods for the isolation of locus MbM1 (see Appendix II) followed procedures outlined in Taylor *et al.* (2002), while those for the isolation of all remaining loci followed Dasmahapatra *et al.* (2004).

### *Parentage analysis*

Of the 23 unique groups studied, 14 were fully sampled (i.e. blood samples were collected and genotypes determined for all adults in the group). Of the five groups containing single unsampled males, paternity assignments were made for three only. In two of these groups the unsampled male was not the dominant bird and is thus unlikely to have sired the offspring. Of the six groups containing unsampled females, maternity assignments were made for three only. Again, in two of these groups the unsampled female was not considered the dominant and is thus unlikely to have been the mother. Where the unsampled individual was considered the dominant male or female in a group, parentage was not assigned.

We only considered individuals sampled within a study site as potential parents for offspring produced within that study site; the terrestrial habits of mesites means that movement between sites was unlikely. However, we considered all adults sampled at one site across all three years as potential parents. Offspring that were produced in 1997 and 1998 were also considered as candidate parents of offspring produced in 1998 and 1999, respectively. In parentage exclusions, one mismatch at one locus per parent-offspring pair was allowed to account for typing errors and

an individual was excluded as putative parent if a mismatch occurred at two or more loci. To obtain the highest reliability for parentage assignment, in addition to the exclusion of genetically incompatible parents, we used the program CERVUS 2.0 (Marshall *et al.* 1998) first to identify the most likely mother and then to identify the most likely father (given the mother) for each offspring. The 'most likely' parent is defined as the one with the largest likelihood ratio. It is calculated by multiplying together the likelihood ratios at each locus (assuming that loci are inherited independently) and is determined relative to the likelihood of parentage of an arbitrary individual (see Marshall *et al.* 1998 for details).

The simulations required by CERVUS for parentage assignments were run with 10 000 cycles, a typing error rate of 0.01 and a sampling proportion of 60% candidate parents. Because the modal number of females and males per group was two and three, respectively, and groups had an average of five neighbouring groups, we used 12 and 18 candidate mothers and fathers, respectively, for the maternity and paternity simulations. The extent of uncertainty in parentage assignments was assessed at 95% (strict) and 80% (relaxed) confidence levels.

In rare cases where only one nonexcluded female/male was inferred to be the most likely mother/father, parentage was assigned to that individual. When two or more individuals were nonexcluded, the most-likely female/male was assumed to be the parent. When all residents were excluded, but a nonresident bird was nonexcluded and was also identified as the most likely parent, that bird was assigned as the mother/father. When two or more genotypic mismatches were detected between all adults and an offspring, parentage was not assigned, irrespective whether or not a parent was identified at the 80% confidence margin. We followed Heckel & von Helversen (2003) in adopting this more parsimonious approach because an important objective of this study was to determine the mating system of the subdesert mesite. The likelihood method potentially allows for 20% erroneously assigned parents (Marshall *et al.* 1998) and actual confidence levels are strongly dependent on the validity of input parameters for the simulation (Constable *et al.* 2001), factors that could lead to misinterpretation of a species' social organization (Vigilant *et al.* 2001).

### *Relatedness analysis*

Genetic relatedness among individuals was calculated using a custom Microsoft Excel Macro 'GROUPRELATE' (Valsecchi *et al.* 2002). This macro performs all-against-all pairwise relatedness values using the equations of Queller & Goodnight (1989) and then partitions the resulting variables by gender within groups. For each class of comparison (e.g. male-female, group P10), the macro then tests whether the average observed relatedness differs

significantly from the mean relatedness calculated from 1000 randomizations wherein original genotypes are replaced with alleles drawn randomly from the observed allele frequency distributions, based on all individuals sampled. This thereby simulates a null situation where no individual is related to any other. As the presence and position of unscored loci were preserved, the number of comparisons in each class remains constant and any biases due to the structure of the data set are allowed for. Relatedness was calculated amongst all individuals in a group, as well as amongst adult birds only (Appendix I).

### *Statistical analyses*

In order to minimize pseudoreplication, groups were defined as unique if new (i.e. unbanded) individuals accounted for more than 50% of the group. For groups that bred in more than one season but whose composition did not change by more than 50% between seasons, data from one season only are provided (e.g. groups M8 and M9). Relatedness values were calculated for each individual, and averaged by sex and within group. Spearman rank correlation tests were used to examine the relationship between seasonal reproductive success and within and between sex relatedness, the sample size being too small to run parametric tests (e.g. a GLM). We pooled data across seasons and sites as there were no significant differences in population sex ratios, mean group sizes, and territory sizes between seasons or sites (Seddon 2001). Seasonal reproductive success was obtained from the 17 and 15 groups with reliable data on chick hatching and offspring survival, respectively. Analyses were conducted using *SPSS* version 11.01, and all tests are two tailed.

## **Results and discussion**

Sequences were obtained for 27 microsatellite-containing clones for which primer pairs were designed. All primer pairs successfully amplified, but only nine were polymorphic with a mean of 5.56 alleles per locus (range: 2–15; Appendix II). The mean observed and expected heterozygosities were 0.469 and 0.517, respectively, and the total exclusionary power using the nine loci was 0.9367 for a first parent and 0.9917 for a second parent (calculated by *CERVUS*). Of the 98 mesites sampled, a mean ( $\pm$  SD) of  $95.6 \pm 4.1$  individuals were typed per locus, and the mean number of loci typed per individual was  $8.8 \pm 0.5$ . Only 78 of the 98 mesites belonged to unique study groups; the rest were floaters or young that dispersed out of the study area.

### *Parentage assignment*

Parentage assignments were made for 22 progeny (Table 1). Mothers were assigned to 15 progeny at the 95% confidence

level and six at the 80% confidence level. The remaining juvenile (GG-SR from group P10) could not be allocated to a mother because there were at least two genotypic mismatches with all candidate females. For five of the offspring, only one candidate female matched at all loci. Of the remaining 17 offspring, at least two females matched the offspring at all loci. In 11 of these cases, one female was identified as the most likely mother at the 95% level and in the remaining six at the 80% level.

Having identified the most likely mothers for all but one offspring, we assigned fathers to eight progeny at the 95% level and 11 at the 80% level. Only two offspring (from groups P6a and M9) had single nonexcluded males, and in both cases these were also the most likely male. However, the unsampled male in group P6a was also the dominant bird and thus likely to be the true father. Therefore, a father was not assigned to this offspring (Table 1). The remaining 20 offspring had two or more nonexcluded males, but as with the mothers, the most likely father identified by *CERVUS* was always among the nonexcluded individuals.

Fifteen progeny (68% of total sampled) had both parents from within their natal group; this figure rises to 17 (77%) if we make the reasonable assumption that the unsampled dominant male in P6a was the father of this group's pair of offspring. Two young had a mother from within their group but their father was from an adjacent territory: the nonresident father of a chick in P8a was a breeding male from P7a, while the father of one of the two chicks produced by M8 was a nonbreeding male from M10 (Table 1). Females were twice observed temporarily visiting neighbouring territories and extra-group copulations may have occurred on these occasions.

Two offspring from M9 had a resident father but an extra-group mother (from neighbouring group M8). Given that egg dumping is unlikely in a species with a clutch size of only two, and juvenile birds do not leave their natal territories before reaching adulthood, the latter observation suggests that the female dispersed or was evicted shortly after reproducing. Dispersal by a breeding female to a neighbouring territory was observed in at least two other groups (P1a and P12a) and may well be a common feature of this species' social system.

### *Social organization and mating system*

The subdesert mesite has a highly variable social and genetic mating system. In our population, 13% of groups were pairs, 17% contained single females with two or more males, 26% contained single males with two or more females and 44% contained multiple males and females. Some of these groups comprised monogamous pairs, while others were polygynandrous (containing two male and female breeders). The lack of evidence of genetic polyandry or polygyny may be an artefact of the small number of

**Table 1** Results of parentage assignments for 22 sampled offspring (chicks and juveniles) by exclusion of putative mothers and fathers and by calculation of the male and female most likely to be the parents (see text). Number of candidate males was 12 at Mangily and 28 at PK32, and number of candidate mothers tested was seven at Mangily and 19 at PK32. Assignments at the 95% confidence level are denoted by asterisks; all other assignments were made at the 80% confidence level. P- and M-prefixes denote study groups at PK32 and Mangily, respectively

Group (year)	Offspring identity	Status†	Nonexcluded females (resident)	Nonexcluded males (resident)	Most-likely mother (group)	Most-likely father (group)	Parental R‡
P1 (98)	S-WG	Chick	1 (1)	3 (1)	W-WS (P1)*	SY-YY (P1)*	<b>0.05</b>
P2a (97)	Y-YS	Juv	3 (1)	7 (2)	R-RS (P2)*	SG-G (P2)	-0.30
P2c (99)	B-S	Juv	3 (1)	3 (1)	G-SR (P2)	SW-R (P2)	-0.17
P4 (98)	S-BR	Juv	4 (2)	3 (1)	Y-SR (P4)	SR-GG (P4)	0.60
P4 (98)	R	Chick	5 (1)	5 (1)	Y-SR (P4)	SR-GG (P4)	0.60
P6 (97)	G-SG	Juv	6 (2)	6 (0)	GW-RS (P6)*	?§	0.10
P6 (97)	SW-R	Juv	3 (1)	1 (0)	GW-RS (P6)*	?§	0.10
P7 (99)	G	Chick	3 (1)	4 (1)	GG-RS (P7)*	SY-R (P7)*	-0.01
P7 (99)	Y	Chick	6 (1)	2 (1)	GG-RS (P7)*	SY-R (P7)*	-0.01
P8a (98)	W-WS	Chick	6 (3)	3 (1)	RR-RS (P8)*	SY-R (P7)*	<b>-0.02</b>
P8a (98)	S-GG	Chick	5 (3)	3 (1)	Y-S (P8)*	S-BB (P8)	<b>0.19</b>
P8b (99)	SB-YY	Chick	3 (1)	6 (2)	Y-S (P8)*	SG-WW (P8)	0.40
P8b (99)	S-RY	Juv	8 (3)	2 (2)	Y-S (P8)	SG-WW (P8)*	0.40
P10 (99)	BB-SW	Juv	1 (1)	5 (3)	RR-SB (P10)*	SY-WW (P10)	0.27
P10 (99)	GG-SR	Juv	1 (0)	7 (4)	?	SW-B (P10)*	?
P12 (99)	B	Chick	11 (1)	2 (1)	RY-S (P12)*	RS-G (P12)	0.09
P12 (99)	BY	Chick	8 (1)	5 (1)	RY-S (P12)*	RS-G (P12)*	0.09
M8 (97)	S-RB	Juv	3 (2)	3 (0)	B-SB (M8)	S-W (M10)	0.22
M8 (97)	S-RG	Juv	4 (2)	4 (2)	R-SR (M8)	SB-W (M8)	<b>-0.05</b>
M9 (97)	SY-G	Juv	1 (0)	1 (1)	B-SB (M8)*	S-RR (M9)	0.49
M9 (97)	YS-W	Juv	1 (0)	2 (1)	B-SB (M8)*	S-RR (M9)	0.49
M11 (97)	BB-SB	Chick	2 (2)	4 (1)	YY-SY (M11)*	SR-R (M11)*	0.38

†Chicks were < 4 weeks old, and were distinguished by downy feathers, a lack of sexually distinct plumage and small size (i.e. < 20 cm in length and < 80 g in mass); juveniles were 4–12 weeks with sex-specific plumage, distinguishable from adults by buffy flanks, supercilia and forehead and lower mass (< 115 g).

‡Bold denotes where relatedness values represent the least related pair in a fully sampled group containing > 1 male.

§Unmarked male in group probably sired offspring.

study groups, as such intermediate mating systems are typically also found in other cooperative breeders exhibiting monogamy and polygyny (e.g. Tasmanian native hen *Gallinula mortierii*, Goldizen *et al.* 1998).

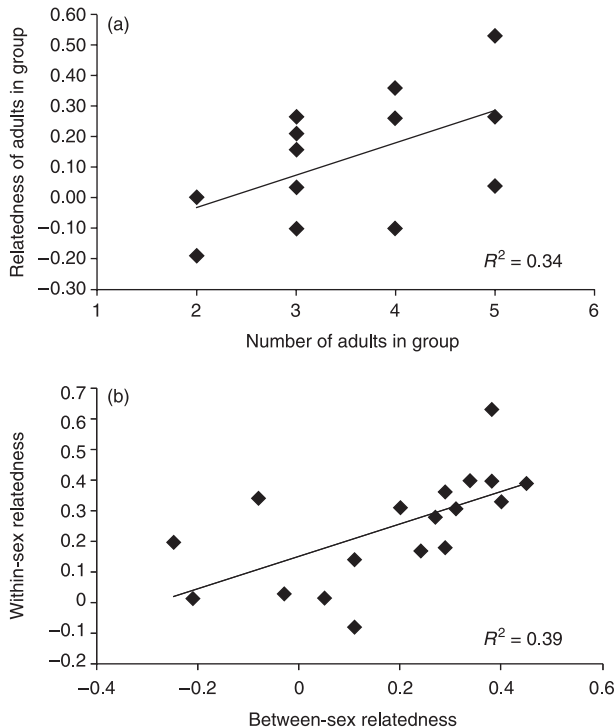
We found that 18 of the 22 progeny consisted of nine pairs of young from the same nest (Table 1). At least five of these had identical mothers and fathers, six if the unsampled male in group P6a was the father of this group's pair of offspring. In other words, genetic monogamy was the most common form of mating system in our study population. However, of the remaining pairs of young, at least two had different mothers and fathers. The final pair of young (from group P10) was sired by two different males and two different females. Both fathers, and at least one female were resident, but the identity of the second mother could not be determined; she may have belonged to the group but dispersed or died prior to the group's capture.

These molecular findings confirm behavioural observations of a dynamic social organization in the subdesert mesite (Seddon *et al.* 2003), making the species similar to

several other avian cooperative breeders, e.g. Tasmanian native hen (Goldizen *et al.* 1998), Arabian babbler *Turdoides squamiceps* (Zahavi 1990) and white-winged chough *Corcorax melanorhamphos* (Heinsohn *et al.* 2000). As in these species, variability in mating arrangements in mesites probably results from the pursuit of sex-specific mating strategies.

#### *Relatedness and dispersal*

The extent to which adult males and females were related to members of their own and opposite sex varied between groups. However, overall, relatedness amongst adults within groups was low to moderate (see Appendix I). For example, of the 14 completely sampled groups, only five comprised adults that were more related to one another than expected by chance across the population as a whole, and in the 10 groups containing more than one male (where all males were sampled) only three contained males that were more related to each other than



**Fig. 1** Correlation between (a) relatedness and the number of adults in a group (Spearman rank correlation:  $r = 0.63$ ,  $P = 0.016$ ,  $n = 14$  groups for which all adult members had been sampled); and (b) within-sex relatedness and between-sex relatedness ( $r = 0.70$ ,  $P < 0.001$ ,  $n = 18$  groups).

expected by chance. Similarly, for the 11 completely sampled multifemale groups, only two contained highly related females.

Large mesite groups contained more related individuals than smaller ones. We found a positive correlation between the number of adults in a group and the degree of relatedness between them (Spearman rank correlation:  $r = 0.44$ ,  $P = 0.041$ ,  $n = 22$  groups), a relationship that was particularly strong when only completely sampled groups were included ( $r = 0.63$ ,  $P = 0.016$ ,  $n = 14$  groups; see Fig. 1a). In contrast, there were no significant relationships between the number of adult females in a group and female relatedness ( $r = 0.014$ ,  $P = 0.966$ ,  $n = 12$ ), nor between the number of adult males in a group and male relatedness ( $r = -0.25$ ,  $P = 0.417$ ,  $n = 13$ ). This suggests that while large groups form by natal philopatry, small groups form via alternative mechanisms, e.g. by siblings dispersing to a vacant territory or by an unrelated helper joining a pair (Seddon 2001). In other words, it appears that different routes to sociality have merged within a single population of subdesert mesites, just as they have in acorn woodpeckers *Melanerpes formicivorus* (Koenig *et al.* 1996), stripe-backed wrens *Campylorhynchus nuchalis* (Rabenold 1990; Piper & Slater 1993) and dunnocks *Prunella modularis* (Davies 1992).

### Reproductive skew and inbreeding

Groups contained coalitions both of related and unrelated helpers: of the 20 groups comprising more than two adults, six contained related helpers and 14 contained unrelated helpers. Further, of the nine groups with multiple offspring, six contained one breeding pair with unrelated helpers and three contained multiple male and female breeders with related helpers (see Appendix I).

Theory predicts a low-skew mating system (i.e. polygyny) where helpers are unrelated (Vehrencamp 1983). However, we found evidence of this in one group only (M8). Group P4 comprised unrelated adults ( $R = 0.12$ ), but only one pair in the group sired the young, whereas in P8 and P10 there was evidence of mixed parentage, yet the groups contained related adults ( $R = 0.36$  and  $0.27$ , respectively).

Such evidence of mixed parentage in highly related groups indicates that incestuous matings might occur in mesites, just as they occur in the closely related rail family (e.g. pukeko *Porphyrio porphyrio*: Craig & Jamieson 1990; moorhen *Gallinula chloropus*: McRae & Burke 1996). However, previous work showed that male mesites with low heterozygosity have smaller territories and lower seasonal reproductive success (Seddon *et al.* 2004). This suggests that the costs of inbreeding are high and should be avoided. Although we found no obvious evidence of this – females bred with males that may have been siblings and mated with the most distantly related male in only 4 of 10 multi-male groups – perhaps other avoidance mechanisms have evolved.

In other cooperatively breeding bird species incestuous mating is minimized by males remaining on their natal territories and females dispersing, e.g. Arabian babblers (Zahavi 1990) and Florida scrub jays *Aphelocoma coerulescens* (Woolfenden & Fitzpatrick 1990). Because the degree to which dispersal is sex biased is reflected by the correlation between within-sex and between-sex relatedness, we evaluated the relationship between these variables. We found it to be positive whether or not offspring were included in the relatedness calculations (including offspring:  $r = 0.70$ ,  $P = 0.001$ ,  $n = 18$  groups; excluding offspring:  $r = 0.56$ ,  $P = 0.024$ ,  $n = 16$  groups; Fig. 1b). This suggests that when philopatry occurs in this species, both males and females are likely to stay.

In the absence of sex-biased dispersal, extra-group paternity (EGP) have instead evolve to minimize inbreeding. The overall frequency of EGP in the study population was 10% (2/20), i.e. 2 of the 20 offspring to which paternity could be assigned had fathers from outside the group (Table 1). This figure is the same as that reported in stripe-backed wrens *Campylorhynchus nuchalis* (i.e. 7/69; Rabenold *et al.* 1990) and close to the 12% (17/137) reported in white-browed scrubwrens *Sericornis frontalis* (Whittingham *et al.* 1997), but is intermediate compared to that of other

Seasonal reproductive success	Relatedness	Statistics		
		<i>r</i>	<i>P</i>	<i>n</i>
Number of chicks fledged	M : M*	0.06	0.847	12
	M : F*	0.21	0.437	16
	F : F*	0.77	<b>0.005</b>	9
	M : M†	0	1	10
	M : F†	-0.19	0.481	16
Number of young surviving > 3 months	F : F†	0.66	0.051	9
	M : M*	-0.03	0.932	10
	M : F*	-0.01	0.988	13
	F : F*	0.67	<b>0.048</b>	9
	M : M†	0.13	0.710	10
	M : F†	-0.08	0.801	13
	F : F†	0.65	0.078	8

**Table 2** Results of Spearman rank correlation tests examining the relationship between seasonal reproductive success and relatedness amongst males (M:M), females (F:F) and between the sexes (M:F). Significant relationships are highlighted in bold

\*Relatedness values for entire group (including offspring).

†Relatedness values for adult group-members only.

cooperative breeders, where it ranges from very low [e.g. 1.3% (1/80) in red-cockaded woodpeckers *Picoides borealis*; Haig *et al.* 1994] to extremely high [e.g. 76% (137/181) in superb fairy wrens *Malurus cyaneus*; Mulder *et al.* 1994]. Although our sample size is small, both cases of EGP in our study occurred in groups with high between-sex relatedness, suggesting that EGP could indeed be an inbreeding-avoidance strategy. However, in only one case did the female breed with an extra-group male that was less related to her than the dominant male in her group ( $R = -0.02$  vs. 0.59). In the second instance, the female chose a male that was more related to her than her social partner ( $R = 0.22$  vs. 0.15).

If female mesites do not avoid incestuous matings, perhaps alternative benefits such as improved genetic quality or compatibility explain why they engage in extra-group fertilizations. For males, however, it is likely that EGPs are sought simply to obtain direct genetic benefits from philopatry. In support of this, one case of EGP in our study population was obtained by a nonbreeding helper from an adjacent territory.

#### *Kinship and reproductive success*

Groups varied in their seasonal reproductive success (Appendix I). Previous work showed that although this variation is unrelated to group size and composition (Seddon *et al.* 2003), it is strongly related to male heterozygosity (Seddon *et al.* 2004). Because kinship influences helping behaviour in species with similarly moderate levels of within-group relatedness (e.g. Seychelles warbler *Acrocephalus sechellensis*; Richardson *et al.* 2003a, b), we examined whether this metric might explain some of the variation in group seasonal reproductive success. We found that although relatedness amongst males and between the sexes was not correlated with reproductive success (Table 2), groups

comprising more related females produced significantly more chicks (relatedness values including offspring:  $P = 0.005$ ,  $R^2 = 0.49$ ; excluding offspring:  $P = 0.051$ ,  $R^2 = 0.43$ ) and had significantly more young surviving to three months of age (including offspring:  $P = 0.048$ ,  $R^2 = 0.45$ ; excluding offspring:  $P = 0.078$ ,  $R^2 = 0.42$ ). In other words, there was a positive correlation between female relatedness and seasonal reproductive success. Of the seven groups for which there were data on helping behaviour, four contained two females; although all the males in these groups incubated eggs, only the dominant female contributed to care (Seddon *et al.* 2003). In all three groups in which both females were sampled, the dominant and subordinate female had fairly low relatedness:  $R = 0.20$ ,  $-0.08$  and  $0.16$  in P2b, P7a and M11, respectively.

Together with the observation that groups consisting of more related females had higher seasonal reproductive success, these findings suggest that help in females may be conditional on kinship, as demonstrated in the Seychelles warbler (Richardson *et al.* 2003a, b). However, three observations suggest that helping in males may be dictated by factors other than kinship: (i) all monogamous groups (i.e. those containing one pair of breeders) had unrelated helpers, (ii) two of the three polygynandrous groups contained highly related adults, and (iii) relatedness amongst males and between males and females had no effect on seasonal reproductive success. The first and the second observations contradict predictions of reproductive skew theory, which states that egalitarian breeding systems (i.e. polygynandry) will arise in groups containing coalitions of unrelated helpers, while monogamy should only occur in highly related groups (Vehrencamp 1983). The lack of support for this basic prediction of skew theory suggests that selective forces in addition to kin selection influence helping behaviour in mesites.

Overall, this study indicates that in the subdesert mesite, cooperative behaviour in females is directed by kinship. In males, however, it may occur because it directly enhances long-term fitness, perhaps by increasing survival and ability to rear offspring in a harsh, arid environment where resources are scarce and predators abundant. This study thereby supports the increasingly popular idea that although kinship is important in the evolution of cooperation, helping behaviour may also arise when group members are unrelated, through a variety of direct benefits (Clutton-Brock 2002).

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## Appendix I

Size, composition and reproductive output of 23 unique groups of subdesert mesites studied in 1997–2000. Values of relatedness ( $R$ ) within and between males and females, including and excluding offspring. Figures in bold ( $P < 0.01$ ) and italics ( $P < 0.05$ ) show where  $R$  within the group is significantly greater than the average  $R$  calculated from 1000 different random pairwise associations across the population as a whole (Mantel tests). P- and M- prefixes denote study groups at PK32 and Mangily, respectively

Group	Season studied (Sept–Jan)	Group composition: no. of adults (proportion sampled)		No. of eggs laid	No. of chicks fledged	No. of young > 3 months	No. of male offspring (proportion sampled)	No. of female offspring (proportion sampled)	Overall relatedness				Adult relatedness			
		Males	Females						M:M	F:F	M:F	All	M:M	F:F	M:F	All
P1a	1998–1999	3 (1)	2 (1)	2	1	1	1 (1)	0	0.17	0.18	0.29	0.23	0.21	0.17	0.29	0.26
P1b	1999–2000	2 (1)	1 (1)	2	2	2	2 (0)	0	0.17	—	0.24	0.22	0.17	—	0.24	0.22
P2a	1997–1998	2 (1)	1 (1)	2	1	1	0	1 (1)	0.46	0.09	0.27	0.27	0.45	0.08	0.26	0.26
P2b	1998–1999	1 (1)	2 (1)	?	?	?	?	?	—	0.20	–0.25	–0.10	—	0.20	–0.25	–0.10
P2c	1999–2000	1 (1)	1 (1)	4	2	2	0	2 (0.5)	—	0.34	–0.08	0.06	—	—	–0.19	–0.19
P3	1999–2000	1 (1)	1 (0)	2	1	1	1 (0)	0	—	—	?	–0.28	—	?	—	–0.28
P4	1998–1999	5 (0.8)	2 (1)	?	3	2	2 (1)	0	0.15	0.47	0.20	0.22	0.11	0.45	0.09	0.12
P5	1998–1999	1 (1)	3 (1)	?	?	?	?	?	—	0.01	–0.21	–0.10	—	0.01	–0.21	–0.10
P6a	1997–1998	1 (0)	2 (1)	2	2	2	1 (1)	1 (1)	—	0.11	?	0.17	0.20	0.08	0.20	0.18
P6b	1999–2000	3 (0.67)	1 (1)	0	0	0	0	0	0.01	—	0.05	0.04	0.01	—	0.05	0.04
P7a	1998–1999	1 (1)	2 (1)	2	?	0	0	0	—	–0.08	0.11	0.05	—	–0.08	0.11	0.05
P7b	1999–2000	1 (1)	1 (1)	2	2	1	2 (1)	0	0.40	—	<b>0.38</b>	<b>0.45</b>	—	—	0.00	0.00
P8a	1998–1999	1 (1)	3 (1)	?	2	?	1 (1)	1 (1)	0.43	<b>0.35</b>	<b>0.45</b>	<b>0.40</b>	—	<b>0.36</b>	0.36	<b>0.36</b>
P8b	1999–2000	3 (1)	2 (0.5)	2	2	2	2 (1)	0	0.36	—	0.29	0.36	0.34	?	?	0.34
P9	1997–1998	3 (1)	2 (0.5)	?	?	?	?	?	0.03	?	–0.03	–0.01	0.03	?	–0.03	–0.01
P10	1999–2000	3 (1)	2 (1)	2	2	2	0	2 (1)	0.37	0.24	<b>0.31</b>	<b>0.31</b>	<b>0.36</b>	0.16	0.23	<b>0.27</b>
P12a	1998–1999	2 (0.5)	2 (0.5)	?	2	?	1 (0)	1 (0)	?	?	–0.31	–0.31	?	?	–0.31	–0.31
P12b	1999–2000	2 (0.5)	1 (1)	?	2	?	1 (1)	1 (1)	0.51	0.74	0.38	0.46	?	—	0.07	0.07
M7	1997–1998	4 (1)	2 (0)	?	?	?	?	?	0.37	?	?	0.37	<b>0.37</b>	?	?	0.37
M8	1997–1998	2 (1)	2 (1)	2	2	2	2 (1)	0	0.34	0.45	<b>0.34</b>	<b>0.35</b>	0.50	0.43	0.15	0.26
M9	1997–1998	3 (1)	2 (0.5)	2	2	2	2 (1)	0	0.14	—	0.10	0.13	0.09	?	0.00	0.04
M10	1997–1998	3 (1)	2 (1)	?	?	?	?	?	—	—	—	—	<b>0.43</b>	<b>0.59</b>	<b>0.55</b>	<b>0.52</b>
M11	1997–1998	1 (1)	2 (1)	2	2	1	0	1 (1)	—	<b>0.33</b>	<b>0.40</b>	<b>0.37</b>	—	0.08	0.21	0.16

\*Unique groups were defined as such if new individuals accounted for > 50% of the group; most groups were caught, bled and ringed in September or October of the study period.

**Appendix II**

Genetic characteristics of nine microsatellite markers used in this study; values are derived using program CERVUS 2.0 (Marshall *et al.* 1998)

Locus	Primers (5'-3')	Repeat	Clone size (bp)	No. of alleles	No. of individuals typed per locus	$H_E$	$H_O$	PIC	Excl(1)	Excl(2)
MbM1	L: GGACAACCTCACCTGAGGAACC R: GTAGGAGAGGATGCAAATCAGG	(CA) <sub>9</sub> AA(CA) <sub>3</sub>	177-198	8	93	0.849	0.826	0.802	0.484	0.658
Mbe2	L: TCAGCACTGGTCTTTGCATC R: ATTTGTGGATGCCAAAATGG	(AAAC) <sub>6</sub>	220	2	91	0.077	0.074	0.071	0.003	0.036
Mbe3	L: TGCCATAAGTGGTGTCTGTGTC R: GATCGTGGTGGTTGTCTTG	(AAAC) <sub>6</sub>	152	2	91	0.209	0.238	0.209	0.028	0.104
Mbe4	L: TCTACAATCTACACCGGATATGG R: CAATGGAAATCAGGGTATTAATTTG	(AAAC) <sub>6</sub>	190	3	90	0.187	0.223	0.200	0.025	0.101
Mbe6	L: TAGGAGTTCAAGCACGAATG R: GATCTGTCTGTTTGTGTGG	(AAAC) <sub>7</sub>	101	4	90	0.538	0.578	0.529	0.178	0.340
Mbe8	L: TCAGGCCTTAGCTATAACCATCC R: GATCACTGTGCAAACTTTGC	(CA) <sub>15</sub>	159	9	93	0.828	0.774	0.737	0.389	0.568
Mbe9	L: AGTCGCCAAGTCTGGAAGTCTG R: AATAGCTTTTGGGCACATCC	(CAA) <sub>6</sub>	318	15	80	0.800	0.901	0.887	0.653	0.790
Mbe12	L: CAGGGATGCTATCATGAGG R: GATCTCATAGCGTTTTTGAAGTCTG	(GTTT) <sub>3</sub> (TTTG) <sub>4</sub>	186	4	93	0.237	0.456*	0.409	0.104	0.239
Mbe13	L: GAAGTGCAACACTTTGTGGAC R: TGTGGAAGTGTTCAGCCTTG	(AAAC) <sub>5</sub>	279	3	93	0.516	0.586	0.518	0.170	0.315

$H_E$ , observed heterozygosity;  $H_O$ , expected heterozygosity; Excl(1), exclusion probability of parent 1; Excl(2), exclusion probability of parent 2; PIC, polymorphic information content; \*significant deviation from Hardy-Weinburg proportions at  $P < 0.001$ .