



Genetic evidence for cooperative polyandry in reverse dichromatic *Eclectus* parrots

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Group size in cooperatively polyandrous birds appears to be limited by the diminishing probability that any one male will achieve paternity as group size increases. Here we use molecular techniques to analyse the mating system of *Eclectus* parrots, a species with a surprisingly large number of males (up to seven) in each group. We show that their social groups are not composed of kin, and that all males seek direct fitness. Most broods of two nestlings had just one father. However, further group males gained paternity in other nesting attempts within and between breeding seasons. Although the likelihood of multiple paternity increased with the number of males in the group, only a subset of group males gained paternity over the eight-year study period. Some males gained paternity at irregular intervals with the same female over multiple years, and many also mated polygynandrously at widely dispersed nests. Our data also confirm that females monopolise scarce breeding hollows over multiple years. We suggest that *Eclectus* parrots occur in larger group sizes than other cooperatively polyandrous species as a result of a severely biased operational sex ratio brought about by a scarcity of breeding females and a male-biased adult sex ratio.

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Polyandry is the rarest of avian mating systems, and occurs when a single female pairs with two or more males during a breeding season (Emlen & Oring 1977; Oring 1982). Polyandry falls into two distinct categories depending on whether the females mate sequentially with single males who then care for the clutch alone (classical polyandry), or with multiple males who care for the clutch cooperatively (cooperative polyandry, Faaborg & Patterson 1981; Oring 1986). Classical polyandry entails sex-role reversal with all or most parental care given by males, and aggressive competition between females for territories and mates. Typically, as soon as one clutch is laid, the male assumes incubation duties and the female seeks to lay further clutches with additional males (Oring et al. 1994; Eens & Pinxten 2000). Although male care of eggs is the likely precursor, the evolutionary ecology of classical polyandry and its restriction to just a few major taxa remain largely unexplained (Andersson 2005).

In contrast, cooperative polyandry occurs when two or more males form stable social units with single females, and some, or all, of the males sire young in a single brood or season. The few studies to date have suggested two types of ecological basis for this behaviour. In some species it appears that groups of males can defend territories better than alone, and that the benefits from sharing paternity can outweigh the costs (e.g. Faaborg & Bednarz 1990; Jamieson et al. 1994; Goldizen et al. 2000). These species often have remarkably nonaggressive interactions between unrelated coalition members. In the second type, space use may differ between the sexes with variable mating systems arising depending on the extent of overlap of male and female territories. In these species, males compete vigorously for mating success, and shared paternity only arises through female control (Davies 1990; Davies et al. 1995; Hartley et al. 1995). In both circumstances, the mating system may become polygynandrous if the males mate with additional females. These females may be part of the same social group and lay in the same nest (e.g. Koenig & Stacey 1990), or maintain separate territories and nests (e.g. Briskie et al. 1998).

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Cooperatively, polyandrous birds generally form smaller groups than species whose cooperative systems are based on the nuclear family (Brown 1987). With one exception (noisy miners, *Manorina melanocephala*, later shown to be monogamous, Poldmaa et al. 1995), Hartley & Davies (1994) showed that breeding groups in cooperatively polyandrous species never had more than three males. They suggested that this occurs because additional males in polyandrous groups face diminishing paternity, whereas additional males in nuclear families have constant relatedness to the brood and are only limited by the extent to which their help increases productivity. They showed with removal experiments that male dunnocks, *Prunella modularis*, preferred to settle in smaller groups, and that females were unwilling to mate with a third male because of the decline in care, and possibly increased harassment, from additional males.

In this paper, we analyse the extent of polyandry in *Electus* parrots, *Electus roratus*, a species in which females and their young may be fed by a surprisingly large number (up to seven) of males (Heinsohn & Legge 2003). *Electus* parrots have long puzzled evolutionary biologists because of their striking reversed plumage dichromatism (red and blue females, green males, Forshaw & Cooper 1989; Amundsen & Parn 2006). However, they are not sex-role reversed as is generally the case for reverse dichromatic birds (Eens & Pinxten 2000). Instead, females have retained the role of incubating eggs and protecting young, and remain unavailable for further mating until their young have fledged. The males, who outnumber females by two to one, compete aggressively for access to them (Heinsohn & Legge 2003; Heinsohn et al. 2005). However, the bright coloration of females does appear to function in intraspecific competition for scarce breeding resources (Heinsohn et al. 2005). Females guard nest hollows in emergent rainforest trees for as long as 11 months each year. Throughout this period, all of their food is provided by the males who forage for fruit over large home ranges (approximately 30 km², Heinsohn & Legge 2003, unpublished data). Multiple matings have been observed in quick succession with little apparent aggression between rival males, but in other cases, males fight vigorously for access to the females. Thus, the mating system has been interpreted as cooperatively polyandrous, and potentially polygynandrous (Heinsohn & Legge 2003) but genetic data on group structure (related versus unrelated males) and the extent of shared paternity have been lacking. Such information is essential as evidenced by the misinterpretation of the mating systems of some social species before genetic data became available (e.g. Poldmaa et al. 1995).

Electus parrots present many logistical challenges in the quest to understand their mating system. These include difficulty in catching the adults and accessing their nest hollows in emergent rainforest trees, and a low rate of reproduction which necessitates data collection over many years for sufficient samples to establish shared paternity. None the less, knowledge of their mating system is essential for understanding their unusual reversed sexual dichromatism, and the large number of males attending females makes them interesting candidates for understanding further the ecology of cooperative polyandry

(and polygynandry) in birds. Here, we use molecular techniques to infer rates of shared paternity on a large temporal and spatial scale. Our data collected over eight years illustrate both the reproductive tensions within polyandrous groups and the nature of individual reproductive rewards over the long term.

METHODS

Study Site and Field Methods

We studied the subspecies *E. r. macgillivrayi* at Iron Range National Park on Cape York Peninsula, in far north Queensland, Australia (12°45'S, 143°17'E). The national park is located in a lowland rainforest of approximately 500 km². *Electus* parrots nest in hollows in emergent rainforest trees from 15 to 30 m above the ground, and virtually always lay two egg clutches. Behavioural studies of banded females have shown long-term and exclusive ownership of each hollow by individual females (Heinsohn & Legge 2003). Our study comprised 33 nest trees with 45 nest hollows: here, we analyse molecular data obtained from females nesting in 34 hollows in 25 nest trees over approximately 50 km². Most nest trees had one hollow ($N = 19$) but some had two ($N = 3$) or three hollows ($N = 3$). The nearest nest trees were 50 m apart, and the furthest were 10.1 km apart. Our study comprised eight breeding seasons from August 1997 until March 2005.

Nest trees were climbed using single rope techniques between five and 11 times each breeding season to establish the breeding status of the female, to gain a small (10–70 µl) blood sample from the brachial vein of each nestling, and to establish the success of the brood. In accordance with Australian National University Animal Experimentation Ethics Committee Permit C.R.E.35.04, potential disturbance to the birds was kept minimal by limiting nest visits to less than 15 min, and nestlings were handled for less than 5 min on each occasion. Nestlings older than six weeks (fledging time = 11–13 weeks) were not handled. Nests were always monitored from a distance to ensure the return of the breeding female. There were no discernible adverse effects of handling the nestlings over the entire study period. There were no nest desertions and females always returned to the nest hollows and resumed incubation or brooding within 20 min of our departure. Weights and survival until fledging were similar in handled and unhandled nestlings. Adult *Electus* feathers left in the hollow by the adult female were also collected for DNA extraction. The regularity of nest checking ensured that these were always recently left by the female. Male *Electus* parrots feed the females only, who then pass food to the nestlings. To assess the number of males feeding females, nests were observed from hides on at least three occasions between laying and fledging. For a full description of field methods, including capture of adults in mist nets, see Heinsohn & Legge (2003).

Molecular analyses

We analysed the mating system of *Electus* parrots based on DNA from 310 *Electus* parrots sampled over eight

breeding seasons, including 18 adult females (nine from blood samples, nine from feathers left in nests), 14 adult males (11 from blood samples, three from feathers from birds found dead), and 278 nestlings (all from blood samples).

DNA was extracted from blood by ammonium acetate extraction (Richardson et al. 2001) after digestion with proteinase K (Progen, Heidelberg, Germany), and from feathers with the EDNA HiSpEx (CHAGA, Perth, WA, Australia) kit. DNA samples were resuspended in ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5–8.0). Sex can be determined for nestlings older than 25 days via the colour of emerging feathers. Nestlings that died before this age were sexed using the molecular technique developed by Griffiths et al. (1998). All samples were genotyped at nine polymorphic microsatellite loci including one sex-linked locus, *Ero01*, as described in Adcock et al. (2005). Loci were run on an ABI 3100 sequencer with an internal size standard and scored using GENEMAPPER 3.7 (Applied Biosystems, Inc., Foster City, CA, U.S.A.).

The difficulty of capturing large canopy-dwelling parrots meant that the proportion of the adult male breeding population captured was insufficient to apply likelihood methods of paternity assignment such as those used in the popular software package CERVUS 3.0 (Marshall et al. 1998). Instead, we assessed maternity directly by exclusion, and rates of shared paternity indirectly by kinship analyses of nestlings as recommended by Jones & Ardren (2003).

Social Structure

We used two techniques to infer the genetic relatedness of group members. First, we used exclusion to disprove parent–offspring relationships for each pair of adult birds in each social group. We then examined whether members of social groups were otherwise more closely related to each other than to other birds in the population using measures of pairwise relatedness (Lynch & Ritland 1999; Wang 2002) and permutation tests. We determined whether females nesting in the same tree were more closely related than those nesting in different trees by examining both the pairwise relatedness of the females themselves and the pairwise relatedness of their offspring (yielding a larger sample size). We tested whether male attendants were related to the females by examining all adult female–adult male pairs, and whether males at the same tree were more closely related to each other (e.g. siblings) by examining all male–male pairs.

We used a permutation test in each case as follows. A mean was calculated of all pairwise relatedness values for individuals at the same tree. The nest tree identifier was then shuffled so that individuals were randomly assigned to a nest tree, and a new mean was calculated. The process of randomizing tree identifiers was repeated 10 000 times, and the original true value of within-tree relatedness was compared against the generated values. We used two-tailed tests and rejected the null hypothesis that relatedness was no higher (or lower) than expected by chance if

the true value was in the highest (or lowest) 2.5% of simulated values.

Hollow ownership and maternal families

We examined whether breeding females had exclusive access to their hollows within and between breeding seasons, and the length of their tenure in years by comparing nestling genotypes with the genotypes of their putative mothers. Putative maternal families were identified whenever a female and at least one nestling were sampled (blood or feather) in the same breeding season. The putative mother was excluded as the actual mother of any nestling with which it did not share at least one allele at every locus. The probability of incorrectly assigning mothers without knowledge of paternal genotypes was calculated following Dodds et al. (1996) incorporating their technique for sex-linked loci. Our eight autosomal loci and one z-linked locus gave exclusion probabilities of 0.978 for male nestlings and 0.955 for female nestlings. Feather samples were genotyped at six autosomal loci and one z-linked locus giving exclusion probabilities of 0.973 for male nestlings and 0.945 for female nestlings.

Paternity

To determine the frequency of multiple paternity, we used Kinship v1.31 (Queller & Goodnight 1989) to classify nestlings within broods and maternal families as either full or half siblings. We used full sibship to infer single paternity and half sibship to determine multiple paternity, based on our analysis that showed by exclusion that females do not lay eggs in nests apart from their own (see Results).

We used two data sets. The first comprised all 99 broods with two nestlings (from 34 hollows over 8 years) regardless of whether the mother had been sampled. The two nestlings in each case were assumed to have the same mother based on our finding that there was no mixed maternity within broods when the putative mother was sampled (see Results). This large data set was useful for determining the overall rate of shared paternity within broods.

The second data set comprised 120 nestlings from 72 broods in 15 maternal families in which the mother had been sampled. These were used to determine the frequency of shared paternity between broods from the same female within breeding seasons, and across multiple years. Error rates for all tests of full and half sibship were calculated by generating 5000 simulated pairs related at each of the levels of kinship in question (half and full sibs). We calculated all values at $P = 0.01$ and 0.05 .

We used similar tests to determine whether males gained paternity at more than one nest hollow in the same breeding season. Pairwise calculations for half sibship were used to determine the frequency of shared paternity between nest hollows. Error rates for all tests were calculated at $P = 0.01$ and 0.05 by generating 5000 simulated half sib pairs.

We performed two analyses to determine whether shared paternity at the same hollow was dependent on the number of observed male attendants. We used

a Generalised Linear Mixed Model (GLMM) to regress the status of each of the 99 two-chick broods (shared or nonshared paternity) against the number of males observed attending the female. The data included repeated measures from individual females over multiple years, so female identity was included as a random factor to avoid pseudoreplication. The analysis was carried out for broods classified as having shared paternity at $P = 0.01$. We used a similar GLMM to examine whether shared paternity between broods from the same female within each breeding season was dependent on the number of male attendants.

RESULTS

Social Structure

There was no evidence that related females preferred to nest in the same tree. There were six trees where multiple females were sampled (five with two females and one with three), yielding a sample of eight female–female pairs for directly testing this hypothesis. In all cases, the pairs were shown not to be mother and daughter by exclusion. Furthermore, the mean relatedness values of -0.00549 (Lynch & Ritland 1999) and 0.13315 (Wang 2002) fell well within the simulated distributions; at the 78.9 and 56.5 percentiles, respectively, confirming that these females were not more closely related than average. Similarly, the larger sample size of these females' offspring (118 nestlings, 73 broods, six nest trees, 15 hollows, 1211 pairwise values) showed no evidence of higher than average relatedness if they were from the same tree. The mean relatedness values of -0.04514 (Lynch & Ritland 1999) and -0.000091 (Wang 2002) fell well within the simulated distributions; at the 18.2 and 45.3 percentiles, respectively.

There was no evidence that males were related to the females they attended. There were six nest trees where at least one adult male and female were sampled. The 12 females and 16 males sampled yielded 38 male–female pairs for analysis. In all cases, these were shown by

exclusion not to be mothers and sons. The mean relatedness values for these data of -0.01975 (Lynch & Ritland 1999) and -0.05729 (Wang 2002) fell well within the simulated distributions; at the 53.3 and 53.5 percentiles, respectively, confirming that males are not more closely related to the females they tend than to other birds in the population.

Similarly, there was no evidence that attendant males were closely related to each other. There were three nest trees where more than one adult male was sampled. The total of 13 males yielded 22 pairs for analysis. In all cases, these were shown by exclusion not to be fathers and sons. The mean relatedness values for these data of -0.01893 (Lynch & Ritland 1999) and -0.04744 (Wang 2002) again fell well within the simulated distributions, at the 72.2 and 43.5 percentiles, respectively, confirming that multiple males attending the same females are not more related on average.

Hollow ownership and maternal families

Our molecular data were consistent with the long-term and exclusive ownership of hollows observed previously in a smaller number of banded females (Heinsohn & Legge 2003). We initially identified 15 putative maternal families. These comprised an adult female and from one to five nestlings all sampled in a single breeding season and all nestlings that shared at least one allele at each locus with the female. In 11 of the maternal families, nestlings hatched in other years were also identified as probable offspring of the known female. High exclusion probabilities allowed us to conclude that the same females bred in each of those hollows over multiple years (Table 1). Five females bred exclusively in their respective hollows over five or more consecutive years (one for eight years, two for seven years, and two for five years).

DNA from feathers recently left in the nest supported this conclusion. In two cases, feather samples were taken from the same hollow in different years; in both cases, the feathers yielded identical genotypes. We concluded that they came from the same individual based on a calculated

Table 1. Maternal families, length of tenure of the sampled female, the number of sires determined by allele counting, and the maximum observed number of male attendants

Maternal family	Female tenure	Broods/nestlings	Sires/year	Sires (all years)	Male attendants	P^*
1	1998–2005	11/21	1–2	4	7	<0.001
2	1997–2001	5/8	1	3	6	0.080
3	1997–1999	4/5	1–2	2	5	0.104
4	2002–2005	4/7	1–2	3	4	0.581
5	2001–2002	2/3	1	1	4	0.063
6	2001–2002	2/4	1	2	4	0.391
7	2001–2005	4/8	1	2	4	0.023
8	1997–2005	12/18	1–2	3	7	<0.001
9	1998–2005	10/15	1–2	3	6	<0.001
10	1998–2000 2003	4/6	1	2	4	0.095
11	2001	1/1	1	1	6	—
12	2001	1/1	1	1	3	—
13	1998	1/2	1	1	1	—
14	1998–2002	8/13	2	3	4	0.096
15	2003	1/2	1	1	4	0.250

*Denotes the probability of getting that number of sires, or fewer, over all years if all male attendants have an equal chance of paternity.

probability of 0.001 of being incorrect (Taberlet & Luikart 1999).

Frequency of Polyandry

Our kinship analysis suggests that at least 8.1% of two nestling broods had two fathers ($P = 0.05$, Table 2). Conversely, at least 36.4% of two nestling broods were fathered by a single male. The data for maternal families showed a higher rate of shared paternity across multiple broods by the same female within a breeding season (32.9%, $P = 0.05$, Table 2).

The likelihood that paternity within a brood was shared increased significantly with the number of males observed attending each female (Wald statistic, $\chi^2_1 = 4.33$, $P = 0.038$, Fig. 1). There was no effect of year of study ($\chi^2_7 = 3.23$), nor was there a significant interaction between number of males and year ($\chi^2_7 = 6.23$). Shared paternity between broods in the same season occurred more frequently and was also significantly dependent on the number of males ($\chi^2_1 = 5.93$, $P = 0.015$, Fig. 1). Again there was no effect of year of study ($\chi^2_7 = 0.76$), nor was there a significant interaction between number of males and year ($\chi^2_7 = 1.86$).

Counting of paternal alleles of nestlings in maternal families showed that within a year the nestlings in each nest were fathered by a minimum of one or two males, and over the whole study up to four males fathered nestlings with the same female (Table 1). The frequency of full sib pairs between years (11.1%, $P = 0.05$) suggested that some males gained paternity with the same females over multiple years. Given the lack of philopatry or relatedness between group members, these closely related offspring were most likely to have resulted from the same males re-mating, rather than their relatives. In one case, a male fathered two offspring at the same nest hollow seven years apart. Other long-term associations where

Table 2. Kinship analyses determining the frequency of full sibs (one father) or half sibs (two fathers) within (a) all two chick broods, and (b) maternal families, within breeding seasons and between years

Data set	Primary hypothesis	P value used	N pairs	% Pairs	
(a) Broods ($N=99$)	Full sibs	0.01	19	19.2	
		0.05	36	36.4	
	Half sibs	0.01	3	3.0	
		0.05	8	8.1	
(b) Maternal families	Within season ($N=73$ pairs)	Full sibs	0.01	9	12.3
			0.05	17	23.3
		Half sibs	0.01	9	12.3
			0.05	24	32.9
	Between years ($N=495$ pairs)	Full sibs	0.01	12	2.4
			0.05	55	11.1
		Half sibs	0.01	71	14.3
			0.05	179	36.2
All pairs ($N=568$)	Full sibs	0.01	21	3.7	
		0.05	72	12.7	
	Half sibs	0.01	80	14.1	
		0.05	203	35.7	

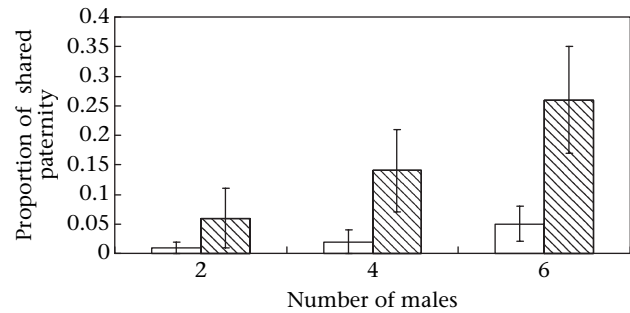


Figure 1. Predicted proportion (\pm SE) of shared paternity for single broods (\square) and multiple broods within the same season (\blacksquare), compared across the number of males observed to feed the female.

the same male fathered offspring with the same female were detected over two ($N = 5$), four ($N = 2$), and five years ($N = 1$).

The data also indicate that many males failed to get any paternity at the nests where they attended females, even when the sample of offspring over eight years was high (Table 1). For example, in the group with the highest number of sampled young (Maternal family 1, $N = 21$ nestlings) only four males achieved paternity when at least seven males were observed to be in attendance. In two other cases where 15 and 18 young were sampled (Maternal families 8 and 9), only three males achieved paternity when at least six were in attendance. If each male had an equal chance of paternity, then the number of fathers observed was significantly lower than expected ($P < 0.05$) in four out of 11 cases where multiple offspring were sampled, or in three out of four cases where more than 10 offspring were sampled from the same female (Table 1). Our data thus suggest that other factors such as female mate choice or unequal competitive abilities of males (e.g. because of age) play a role in determining how many males gain reproductive success.

Frequency of polygyny

Our data also confirmed that many *Ecliptus* parrots mated polygynandrously. Kinship analyses showed that between 11.1 and 58.3% of nestlings in each year had a half sibling in another nest (Table 3). The proportion of successful males that gained paternity in a second nest was highly variable between years, and ranged from 10 to 85%. Seven of the 63 half sibships detected were from hollows in the same tree and 56 were from different trees. Distances between these trees ranged from 500 to 7226 m (mean = 3503 m \pm 260 SE). The long period between egg laying and fledging of young (almost four months), combined with repeated nesting attempts by each female, led to much overlap between nesting attempts across the population. Over half (34) of the half sibships detected involved nests that were active simultaneously, confirming that males often gained paternity at synchronous nests. However, the low number of known individual males made it difficult to determine if nest

Table 3. Incidence of polygynandrous mating as detected by half sibships ($P = 0.01$) between nests for each year of the study

Year	Half sib pairs	%	N (nestlings)*	%	N (nests)†	%	Polygynandrous males (%)‡
1997	1/104	1.0	2/16	12.5	2/11	18.2	10
1998	25/1375	1.8	35/60	58.3	23/25	92.0	62–85
1999	2/376	0.5	4/32	12.5	4/18	22.2	11–13
2000	3/526	0.6	6/35	17.1	6/21	28.6	17
2001	18/1650	1.1	30/59	50.8	19/25	76.0	50–67
2002	3/664	0.5	5/33	15.2	4/19	21.1	11–12
2003	10/402	2.5	13/30	43.3	10/15	66.7	46–50
2004	1/135	0.7	2/18	11.1	2/11	11.1	10

*The number of nestlings with a half sib at another nest.

†The number of nests in which at least one nestling produced in that season had a half sib in another nest.

‡The estimated percentage of breeding males that gained paternity at a second nest.

synchrony affected their provisioning behaviour and investment at each nest.

DISCUSSION

Eclactus parrots differ from many cooperatively breeding species in that their social groups are not kin-based and individuals do not help others to breed for inclusive fitness benefits (Brown 1987; Cockburn 1998; Dickinson & Hatchwell 2004). Females nesting in the same tree were not relatives, and the males that attended them were not their grown offspring or related to each other. Thus, all males appear to be seeking direct mating privileges. Although we could not obtain samples from all males, our indirect techniques none the less provide strong evidence that paternity at nests is often shared, and that males may gain paternity, simultaneously or sequentially, at more than one nest each season. Many of these nests are separated by large distances (up to 7.2 km).

Our data also provide important confirmation of the exclusive long-term use of nest hollows by some female *Eclactus* parrots. This trend was observed previously in banded females over a shorter time-span, but was based on behavioural evidence only. Our genetic analysis shows that 11 out of 15 sampled females held their hollows continuously from two to eight years, and were the only females to use those hollows over those periods. We have previously argued that nest hollows are a scarce and exclusively guarded resource in this species with important implications for both their social system and unusual coloration. Females do not leave their nest trees for up to 11 months each year, and during this time repel (and sometimes kill) intruding females. An analysis of the function of colour suggested that bright red plumage evolved independently in females to signal ownership of scarce hollows (Heinsohn et al. 2005).

Female *Eclactus* parrots may be fed at the nest hollow by a surprisingly large number of males. Most females in this study had more than one male attendant, and two of the females with longest tenure at their hollows (seven and eight years, Table 1) were fed at the nest by at least seven males. However, paternity was not shared equally amongst all group members, even though the extent of

shared paternity (within and between broods) increased significantly with the number of male attendants. Instead, females tended to mate repeatedly with the same subset of males over multiple years, with some males apparently fathering offspring several (up to seven) years apart. All females had only one or two mates each season, and even the largest sample of 21 offspring from one female over seven years had just four fathers when at least seven males were in attendance (Table 1).

In their analysis of cooperative polyandry, Hartley & Davies (1994) suggested that male coalitions should not be too large because of the dilution of paternity and the increased harassment of females when too many males compete. In support of their argument, they showed that male dunnocks preferred to join groups with only one male and, importantly, that females would mate with a second male but were disinclined to mate with a third male. Thus groups with three or more males were unusual and only occurred when the operational sex ratio was severely male-biased.

The unusually large group sizes observed in *Eclactus* parrot groups are probably caused by permanently male biased operational sex ratios. Two factors are known to bias the operational sex ratio towards males in this species. First, as discussed above, use-able nest hollows are a scarce and intensely disputed resource. The shortage of hollows leaves some mature females unable to breed. Second, the adult sex ratio is heavily biased (approximately 67%) towards males, probably because of differential mortality of juvenile females (Heinsohn & Legge 2003). The shortage of females may leave males no option but to join large groups even if their chance of mating with the female is low. Interestingly, we have observed females mating with up to four males in quick succession suggesting that the optimal group size from the female's perspective may be higher than that observed in dunnocks and other cooperatively polyandrous species. However, the clutch size (two eggs) is too small for all *Eclactus* males that mate to father nestlings immediately; instead they appear to accumulate reproductive rewards over the longer term. Our data show that males may father offspring with the same female sporadically over several (up to seven) years. Such long-term rewards are rarely shown and appear to be necessary to explain cooperative polyandry in long-lived

species with small clutch sizes (e.g. Millar et al. 1994). However, our long-term data also suggest that some male *Electus* parrots may achieve very little reproductive success even if they join groups and feed the females. Our inability to establish whether these males attend females for many years before gaining paternity, or simply never gain reproductive success, highlights the difficulty of working with long-lived species such as parrots.

In most of the cooperatively polyandrous species studied to date, males appear to benefit from group-territoriality to the extent that the long-term benefits outweigh the costs of shared paternity (e.g. Faaborg & Bednarz 1990; Koenig & Stacey 1990; Jamieson et al. 1994; Sherman 1995). However, male *Electus* parrots do not defend territories, even though they compete aggressively for access to breeding females (Heinsohn & Legge 2003). Instead, their variable mating system shows some similarities to that seen in dunnocks (Davies 1992) and alpine accentors, *Prunella collaris* (Davies et al. 1995), albeit on a larger spatial scale. In dunnocks, the sexes follow different rules when defending territories, with each male defending an area that encompasses as many females as possible, and females only defending the resources they need for breeding. In accentors, the males do not defend territories but their large home ranges may none the less encompass many females. Thus, in both species, males occupy relatively large home ranges or territories that may encompass multiple smaller female territories.

The disparity in space use by male and female *Electus* parrots is even more extreme. Females stay at their nest hollows for most of the year, and are totally dependent on males to provide food (Heinsohn & Legge 2003). Males, by contrast, have large overlapping home ranges up to 30 km², and each male's range encompasses the nests of many females (range 3–18, unpublished data). Like accentors, such large areas may be impossible for the males to defend, and they may also be limited by having to travel large distances to obtain fruit for the females. Unlike accentors, however, mate guarding has not been observed. Instead, large numbers of males converge on each female when she becomes fertile, leading to increased male–male competition at the nest hollow. Like dunnocks and accentors, female *Electus* parrots probably use copulations to encourage multiple males to attend them (Davies 1992; Hartley et al. 1995). The male-biased sex ratio appears to work in the females' favour as they mate with and attain more male attendants than there are opportunities for paternity (Heinsohn & Legge 2003). Some males none the less realize the potential for multiple mates afforded by overlapping space, as many in this study gained paternity at more than one nest tree.

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References

- Adcock, G. J., Heinsohn, R., Ebert, D., Amini, N. & Peakall, R. 2005. Microsatellite loci for behavioural studies of *Electus* parrot (*Electus roratus*: Aves). *Molecular Ecology Notes*, **5**, 616–618.
- Amundsen, T. & Parn, H. 2006. Female coloration: a review of functional and non-functional hypotheses. In: *Avian Coloration* (Ed. by G. E. Hill & K. J. McGraw), pp. 280–348. Boston: Harvard University Press.
- Andersson, M. 2005. Evolution of classical polyandry: three steps to female emancipation. *Ethology*, **111**, 1–23.
- Briskie, J. V., Montgomerie, R., Poldmaa, T. & Boag, P. T. 1998. Paternity and paternal care in the polygynandrous Smith's longspur. *Behavioral Ecology and Sociobiology*, **43**, 181–190.
- Brown, J. L. 1987. *Helping and Communal Breeding in Birds*. Princeton: Princeton University Press.
- Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, **29**, 141–177.
- Davies, N. B. 1990. Dunnocks: cooperation and conflict among males and females in a variable mating system. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behaviour* (Ed. by P. B. Stacey & W. D. Koenig), pp. 455–485. Cambridge: Cambridge University Press.
- Davies, N. B. 1992. *Dunnock Behaviour and Social Evolution*. Oxford: Oxford University Press.
- Davies, N. B., Hartley, I. R., Hatchwell, B. J., Desrochers, A., Skeer, J. & Nebel, D. 1995. The polygynandrous mating system of the alpine accentor, *Prunella collaris*. 1. Ecological causes and reproductive conflicts. *Animal Behaviour*, **49**, 769–788.
- Dickinson, J. L. & Hatchwell, B. J. 2004. Fitness consequences of helping. In: *Ecology and Evolution of Cooperative Breeding in Birds* (Ed. by W. D. Koenig & J. L. Dickinson), pp. 48–66. Cambridge: Cambridge University Press.
- Dodds, K. G., Tate, M. L., McEwan, J. C. & Crawford, A. M. 1996. Exclusion probabilities for pedigree testing farm animals. *Theoretical and Applied Genetics*, **92**, 966–975.
- Eens, M. & Pinxten, R. 2000. Sex-role reversal in vertebrates: behavioral and endocrinological accounts. *Behavioural Processes*, **51**, 135–147.
- Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215–223.
- Faaborg, J. & Bednarz, J. C. 1990. Galapagos and Harris' hawks: divergent causes of sociality in two raptors. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (Ed. by P. B. Stacey & W. D. Koenig), pp. 359–383. Cambridge: Cambridge University Press.
- Faaborg, J. & Patterson, C. B. 1981. The characteristics and occurrence of cooperative polyandry. *Ibis*, **123**, 477–484.
- Forshaw, J. M. & Cooper, W. D. 1989. *Parrots of the World*. Willoughby: Lansdowne Press.
- Goldizen, A. W., Buchan, J. C., Putland, D. A., Goldizen, A. R. & Krebs, E. A. 2000. Patterns of mate-sharing in a population of Tasmanian native hens *Gallinula mortierii*. *Ibis*, **142**, 40–47.

- Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. 1998. A DNA test to sex most birds. *Molecular Ecology*, **7**, 1071–1075.
- Hartley, I. R. & Davies, N. B. 1994. Limits to cooperative polyandry in birds. *Proceedings of the Royal Society of London, Series B*, **257**, 67–73.
- Hartley, I. R., Davies, N. B., Hatchwell, B. J., Desrochers, A., Nebel, D. & Burke, T. 1995. The polygynandrous mating system of the alpine accentor, *Prunella collaris*. 2. Multiple paternity and multiple effort. *Animal Behaviour*, **49**, 789–803.
- Heinsohn, R. & Legge, S. 2003. Breeding biology of the reverse-dichromatic, co-operative parrot *Eclectus roratus*. *Journal of Zoology (London)*, **259**, 197–208.
- Heinsohn, R., Legge, S. & Endler, J. A. 2005. Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science*, **309**, 617–619.
- Jamieson, I. G., Quinn, J. S., Rose, P. A. & White, B. N. 1994. Shared paternity among non-relatives is the result of an egalitarian mating system in a communally breeding bird, the pukeko. *Proceedings of the Royal Society of London, Series B*, **257**, 271–277.
- Jones, A. G. & Ardren, W. R. 2003. Methods of parentage analysis in natural populations. *Molecular Ecology*, **12**, 2511–2523.
- Koenig, W. D. & Stacey, P. B. 1990. Acorn woodpeckers: group-living and food storage under contrasting ecological conditions. In: *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (Ed. by P. B. Stacey & W. D. Koenig), pp. 413–453. Cambridge: Cambridge University press.
- Lynch, M. & Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753–1766.
- Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- Millar, C. D., Anthony, I., Lambert, D. M., Stapleton, P. M., Bergmann, C. C., Bellamy, A. R. & Young, E. C. 1994. Patterns of reproductive success determined by DNA fingerprinting in a communally breeding oceanic bird. *Biological Journal of the Linnaean Society*, **52**, 31–48.
- Oring, L. W. 1982. Avian mating systems. In: *Avian Biology*. Vol. 6 (Ed. by D. S. Farner, J. S. King & K. C. Parkes), New York: Academic Press.
- Oring, L. W. 1986. Avian polyandry. In: *Avian Biology* (Ed. by R. J. Johnston), pp. 309–351. New York: Plenum.
- Oring, L. W., Reed, J. M. & Alberico, J. A. R. 1994. Mate acquisition tactics in polyandrous spotted sandpipers (*Actitis macularia*): the role of age and experience. *Behavioral Ecology*, **5**, 9–16.
- Poldmaa, T., Montgomerie, R. & Boag, P. 1995. Mating system of the cooperatively breeding noisy miner *Manorina melancephala*, as revealed by DNA profiling. *Behavioral Ecology and Sociobiology*, **37**, 137–143.
- Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.
- Richardson, D. L., Jury, F. L., Blaakmeer, K., Komdeur, J. & Burke, T. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). *Molecular Ecology*, **10**, 2263–2273.
- Sherman, P. T. 1995. Social organization of cooperatively polyandrous white-winged trumpeters (*Psophia leucoptera*) in Peru. *Auk*, **112**, 296–309.
- Taberlet, P. & Luikart, G. 1999. Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnaean Society*, **68**, 41–55.
- Wang, J. L. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics*, **160**, 1203–1215.