Behavioral Ecology Vol. 15 No. 6: 907–915
doi:10.1093/beheco/arh093
Advance Access publication on June 16, 2004

Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction

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Extrapair fertilizations complicate our understanding of cooperative breeding in a number of ways. For example, auxiliaries may reduce the costs of seeking extrapair fertilizations for breeding males or females, and auxiliary males may themselves seek copulations with the breeding female in their own group. We employed microsatellite markers to examine patterns of parentage in the cooperatively breeding splendid fairy-wren (*Malurus splendens melanotus*). Our study population exhibited a relatively high level of extrapair paternity (42% of 386 offspring) with considerable annual variation (range = 24–52%). Across years the proportion of offspring sired by extrapair males was significantly correlated with the average number of auxiliaries per group. Furthermore, the proportion of extrapair young within a brood was related to group composition; groups with multiple auxiliaries were twice as likely as groups with zero or one auxiliary to contain extrapair young. Most offspring were sired by dominant breeding males, but auxiliary males sired approximately 25% of all extrapair young (10% of all offspring), and about half of these were cases in which the auxiliary male sired offspring in his own group. Within-group sirings by auxiliary males were most common after replacement of the breeding female, and they also appeared to be more likely when the auxiliary was not related to the breeding male. Thus, the presence of auxiliary males increased the likelihood that females would produce extrapair young, and although incest avoidance mechanisms usually prevent within-group copulations by auxiliary males, a conflict of interest among group males arises when a new female joins the group. *Key words:* cooperative breeding, extrapair reproduction, *Malurus splendens*, microsatellites, reproductive skew, splendid fairy-wren. *[Behav Ecol 15:907–915 (2004)]*

Cooperative breeding is a social system in which breeding individuals are assisted by other individuals, often termed "auxiliaries," in raising offspring (Emlen, 1991). In many cases, auxiliaries are offspring (or close kin) of the breeding pair that are constrained from independent breeding by ecological and/or social factors (reviewed in Emlen, 1991, 1997; Jennions and MacDonald, 1994). Recent studies indicate genetic monogamy in many cooperative systems (Bruce et al., 1996; Dickinson et al., 1995; Haig et al., 1994; Quinn et al., 1999), an important consequence of which is high relatedness between auxiliaries and the young that they help raise. Thus, auxiliaries may increase their inclusive fitness by assisting breeders (Griffin and West, 2003).

Recently it has become clear that a number of cooperative systems do not fit this simple picture (Clutton-Brock, 2002). In particular, reproductive promiscuity occurs in many cooperative systems (e.g., Hatchwell et al., 2002; Haydock et al., 1996; Jamieson et al., 1994; Joste et al., 1985; Lundy et al., 1998; Rabenold et al., 1990; Wrege and Emlen, 1987) and may be particularly common in some (Brooker et al., 1990; Li and Brown, 2000; Mulder et al., 1994; Whittingham et al., 1997).

Promiscuity complicates our understanding of cooperative breeding in several important ways. First, promiscuity can affect the Hamiltonian cost/benefit analysis for helping behavior. For example, extrapair fertilizations (EPF, fertilizations resulting from copulations between females and any male other than her social mate, including auxiliary males within the group) can affect the indirect benefits of helping by reducing relatedness between auxiliaries and the young that they help to raise (e.g., Clutton-Brock, 2003; Dunn et al., 1995; Richardson et al., 2002). Auxiliaries also may be able to reproduce and thereby accrue direct fitness benefits to partially balance the costs of helping. This can occur if auxiliary males copulate with breeding females in their own group or in other groups, or if auxiliary females lay eggs surreptitiously in the nests of breeding females. Recent studies have suggested that the fitness benefits of reproduction by auxiliaries can be substantial (Baglione et al., 2002; Li and Brown, 2000; Magrath and Whittingham, 1997; Richardson et al., 2002; Whittingham et al., 1997).

Second, in complex social systems like those of cooperative breeders, individual breeding strategies might be affected by group composition. For example, changes in group composition might affect genetic relatedness among opposite-sex group members and alter the dynamics of family relationships. Conflicts of interest are expected in most social groups (Cockburn, 2003; Emlen, 1997) but should be particularly acute in systems with high levels of EPF. In particular, if the breeding female is replaced through death or divorce, relatedness between auxiliaries and the breeding female will change and both the breeding male and auxiliary males may seek copulations with the new breeding female (Emlen, 1995). Group composition might also affect the ability of individuals to seek extrapair copulations. Specifically, breeding females in groups with auxiliaries may be more likely to seek EPF because auxiliaries can compensate for reduced male parental care should it occur (Mulder et al., 1994). Similarly, breeding males

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Received 4 July 2003; revised 8 January 2004; accepted 16 January 2004.

in groups with auxiliaries might also be better able to pursue EPF because they are liberated from parental duties (Green et al., 1995).

Thus, there is now a need to better understand the causes and consequences of reproductive promiscuity in complex social systems. Fairy-wrens (Maluridae) have emerged as a model group for studying both cooperative breeding (e.g., Dunn et al., 1995; Pruett-Jones and Lewis, 1990; Rowley and Russell, 1990b) and reproductive promiscuity (e.g., Brooker et al., 1990; Dunn and Cockburn, 1998; Karubian, 2002; Mulder et al., 1994). In this study we used single-locus microsatellite markers to examine parentage across six breeding seasons for a South Australian population of splendid fairy-wrens (*Malurus splendens melanotus*). Splendid fairy-wrens breed cooperatively and exhibit high levels of reproductive promiscuity (Brooker et al., 1990; Rowley and Russell, 1997). Here we describe year-to-year variation in parentage patterns and the identities of extrapair sires (for the first time in this species), and we use these data to address three issues of interest regarding the mating strategies of breeders and auxiliaries in cooperative breeding systems. First, we demonstrate geographical variation in the incidence of EPF within a single species. Second, we test the hypothesis that the presence of auxiliaries facilitates extrapair mating by the breeding male and/or the breeding female (Green et al., 1995; Mulder et al., 1994). Third, we test the hypothesis that within-group reproduction by auxiliaries is limited by their relatedness to the dominant breeders within the group (Emlen, 1997). This hypothesis predicts that reproduction by male auxiliaries should increase when they are unrelated to the breeding female, because fitness costs associated with inbreeding are removed. It predicts also that reproduction by male auxiliaries should increase as their relatedness to the breeding male decreases, because dominant males are forced to concede some amount of reproduction to retain unrelated helpers within the group.

METHODS

Study species and field methods

This research was carried out during the austral spring (early October to late December) each year from 1992 to 1998 at Brookfield Conservation Park (BCP), approximately 100 km northeast of Adelaide, South Australia. This site is characterized by mallee (*Eucalyptus*) scrub forest and chenopod shrublands, with rainfall averaging 330 mm per year (1992–1997 average). Splendid fairy-wrens are common and abundant in the scrub forest at this site, occurring sympatrically there with the variegated fairy-wren (*M. lamberti*). A third species of malurid, the white-winged fairy-wren (*M. leucopterus*), is found in the chenopod shrublands at BCP. Additional details of the study site are provided in Tibbetts and Pruett-Jones (1999) and Van Bael and Pruett-Jones (2000).

During each field season, fieldwork was conducted on a daily basis. We studied between 35 and 69 family groups each year. By definition, all groups contained at least one male and one female. The mean group size in each year ranged from 2.4 to 3.1 (i.e., the breeding pair plus 0.4 to 1.1 auxiliaries), with 30% to 62% of these groups having at least one auxiliary. We individually color-banded and collected blood samples from most adults in the population, and nestlings were banded and bled at four to eight days of age. For each family group we delineated territorial boundaries, monitored group composition during the breeding season, and located and followed the fate of each nest. Determination of the social status of males in each group was generally easy, based on behavioral interactions (aggressive chases), age, and plumage variation. Breeding by splendid fairy-wrens at BCP is highly concentrated between October and December (Van Bael and Pruett-Jones, 2000), although some nesting is likely to have occurred in January, when we were not at the field site. Our samples of offspring therefore represent the vast majority, but not all, of the offspring produced by this population.

Auxiliaries were generally young males that remained in their natal groups for one or more years. Few females remained in their natal groups after the start of the breeding season following their birth; of 319 groups studied over the six years, 286 (89.7%) had just one female. Moreover, although plural breeding (i.e., breeding attempts by more than one female in a group) occurs in this species (Rowley et al., 1989), it was very rare in our study population—we observed only one case of attempted plural breeding between 1992 and 1998 (Van Bael and Pruett-Jones, 2000).

Given that the female and the dominant male in each group form a strong, social pair bond with each other, we use the term "extrapair" to refer to interaction (copulation or fertilization) by the female and a male other than her social mate. Thus, extrapair copulations or fertilizations can occur between females and any male (dominant or auxiliary) outside her group or between a female and an auxiliary male within her group (see Emlen et al., 1998; Li and Brown, 2000). If males sire offspring in groups other than their own, we refer to these as "extragroup fertilizations."

Development of microsatellite markers

We used the method of Hammond et al. (1998) to construct an *M. splendens* genomic library enriched for simple sequence repeats. This procedure utilized a hybridization technique to create a population of genomic fragments that was enriched for CA and GA repeats. We ligated 5–15 ng of the enriched fragment population into 100 ng of a pUC 18 vector that had been linearized with *BamHI* and dephosphorylated (Ready-to-Go™ pUC 18, Pharmacia) following manufacturer protocols. We transformed 4 μl of the ligation products into XL-2 Blue MRF' Ultracompetent Cells (Stratagene), again following manufacturer protocols, and we grew the transformants overnight at 37°C on LB agar plates containing 80 μg/ml X-Gal, 20 mM IPTG, and 100 μg/ml ampicillin. White colonies (i.e., those containing bird DNA) were picked with a sterile toothpick and replated. We performed standard colony lifts to transfer DNA from these new plates to Nytran® circular nylon membranes (Schleicher and Schuell). To detect colonies containing dinucleotide repeats, we probed the colony lifts non-radioactively with $(GA)_{12}$ and $(CA)_{12}$ oligonucleotides using the ECL™ kit (Amersham). For this probing, oligonucleotide probes were end-labeled with flourescein-dUTP and hybridized to membranes at 42°C under conditions specified in the manufacturer's protocols. After hybridization, the membranes were washed according to recommended protocols and exposed to autoradiography film for 15–60 min.

We amplified and sequenced genomic DNA from an arbitrary subset of colonies that probed positively. Most of these contained long GA or CA repeats. We used Oligo 4.0 (NBI) to design PCR primers that flanked the microsatellite sequence in each of several clones. After initial tests to optimize PCR conditions, we tested each locus for polymorphism by amplifying DNA from approximately 10 individual *M. splendens*. For this screening, we added 1 μl of suspended DNA (approximately 50 ng) to a 25 μl mixture containing 0.15 mM dNTP (each), 0.50 μM each primer, and our standard PCR mixture (1–3 units Taq DNA polymerase, 3.0 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl). Following an initial 3 min denaturation at 94°C, the reaction mix went through 30 cycles of 94°C for 60 s, *X*°C for 60 s, and 72°C for 45 s, where *X*

was the optimized annealing temperature. We checked amplification on a 2% agarose/TBE gel. We used a procedure identical to that of Double et al. (1997b) to test loci isolated from the genome of superb fairy-wrens (*M. cyaneus*). Loci showing high polymorphism and repeatability were selected for genotyping analyses.

Genotyping individuals

A total of six microsatellite loci were selected for genotyping analyses (Table 1)—three isolated from our *M. splendens* library (*Msp4, Msp6, Msp10*; deposited in Genbank, accession numbers AY320050-AY320052) and three loci (*Mcy3, Mcy7, Mcy8*) reported by Double et al. (1997b). We used two different procedures to determine individual genotypes. For samples collected during the 1992–1995 field seasons, we amplified genomic DNA from each individual in a 10 μl PCR reaction that contained 100 μM dNTP (each), 0.25 μM primers (each), 1.5 μCi 33-P dATP (NEN™ Life Science Products), and our standard PCR reaction mix. This reaction was cycled as for the initial screening reactions (above). The PCR products were electrophoresed in a 6% denaturing polyacrylamide gel containing 7M urea at a constant temperature of 50°C. We also ran a standard sequencing reaction of M13 DNA as a size reference for each set of PCR reactions run on a gel. After electrophoresis, the gel was dried and exposed to autoradiography film for 1–3 days. We scored the size of PCR fragments for each individual by comparing its band(s) to the reference M13 sequence.

For samples collected during the 1996–1998 breeding seasons (and a few from earlier years), PCR protocols were similar to those in the screening procedure described above, except that the forward primer from each pair was labeled with a flourescent dye (6-FAM, HEX, or TET; Perkin-Elmer) on the 5′ end, and the annealing temperatures were increased for some loci (Table 1). In some cases, we also reduced the concentration of primers to reduce the production of primer-dimers. After PCR, products from one to three loci labeled with different dyes, or those from loci that did not overlap in fragment size, were combined with sterile water, formamide, and a size standard (ROX 350 or ROX 500; Perkin-Elmer) for separation in a 4% acrylamide gel using an ABI 377 Automated Sequencer (Perkin-Elmer). Fragment sizes were calculated with GENESCAN™ software (Applied Biosystems) and evaluated manually.

The observed allele sizes of the five dinucleotide repeat loci generally matched our expectations based on repeat unit size. However, the tetranucleotide repeat locus *Mcy8* showed a number of alleles that were of unexpected size (i.e., one or two bases, rather than four, from the nearest allele). These unusual alleles occurred in three different regions of the allele size distribution. Therefore, when scoring genotypes we "binned" allele sizes to force most alleles into sizes expected from variation in number of repeat units (alleles that did not exactly match an expected size were assigned to the nearest appropriate size). There were four "odd" allele sizes that we accepted (did not bin) because they were common, intermediate between the nearest expected sizes, and consistent across gels. We also binned all alleles larger than 359 bp at this locus (i.e., all alleles larger than this were treated as the same allele), as we had difficulty detecting small mobility differences in this region of our gels.

Finally, because we used two different procedures to determine individual genotypes, we calibrated the two procedures by using both methods on a subsample of individuals ($n = 19$ to 75 individuals per locus). All loci showed either identical scores, or a small systematic difference that allowed for simple adjustment to align scores.

Table 1
Microsatellites used for genotyping analyses

Locus	PCR Primers (5′ → 3′)	T_a (°C) (1)	T_a (°C) (2)
Msp 4	GGAGAGACCGGGAAACAGAGAC GCAGCACCCTTGGGACGCTCAT	60	65
Msp 6	GCAGGTTTTTAATGGCATCAAG GGAGCTCAAAACTTTAGAATGA	60	60
Msp 10	CGCGTCAAATAAGGGGGAAACC GCAGCCAGCGCCAACAGAAACG	62	65
Mcy 3	ACAAAGGCAAACCTACCCAC TTTTTTTCAAGCGTGCATTC	55	55
Mcy 7	CTTTGTGTTGCTGTTAGGTAGAA GGCTCAACAGCTATTTGCAT	55	62
Mcy 8	CCCAATGGTGATGAAAGTCC ACATTAGTCTTCCCTTTTTTTCC	55	62

Msp loci were isolated in this study, and *Mcy* loci were from Double et al. (1997b). All microsatellites were dinucleotide (CA or GA) repeats, except *Mcy 8*, which was a tetranucleotide (AAAG) repeat. "T_a" is the optimized annealing temperature for PCR reactions with radio-labelled dATP (1) and dye-labelled primers (2).

Determination of parentage

For each locus, we determined the frequency of each allele (x_i), the expected frequency of heterozygotes (h_e, from Equation 8.4 in Nei, 1987) and the observed frequency of heterozygotes (h_o). Observed numbers of heterozygotes were compared to expected numbers using a standard goodness-of-fit test with only two classes (homozygotes and heterozygotes), significance level adjusted for multiple tests (Bonferroni adjustment), and a continuity correction suggested for Hardy-Weinberg tests (Lessios, 1992). A significant difference between h_e and h_o suggests the presence of null (i.e., non-amplifying) alleles (Pemberton et al., 1995), the frequencies of which were determined using the procedure of Summers and Amos (1997). We also calculated three different exclusion probabilities for each locus. First, we calculated the average probability of excluding a randomly chosen female as the mother (i.e., the probability that the female would not possess one of the offspring's alleles at the locus in question). Second, we calculated the average probability of paternal exclusion for each locus following Jamieson (1994). This is the probability, averaged over all alleles at the locus, that a randomly chosen male (i.e., a non-sire) will not possess the paternal allele found in an offspring, given that the mother of the offspring is known with certainty. Finally, we calculated the probability of excluding non-sires that were related to the true sire using the equations presented in Double et al. (1997a).

The above calculations were used to characterize each microsatellite locus and also to serve as a rough guide to the power of our parentage analyses. However, inferences based on exclusion probabilities sometimes can be misleading (Marshall et al., 1998), particularly when many potential sires are closely related to each other (Double et al., 1997a). Therefore, we used the program *CERVUS* 1.0 (Marshall et al., 1998), which uses a likelihood approach to assign parentage. For these analyses we assumed that each breeding female was a biological parent of the nestlings in her own nest, and we assessed the validity of this assumption by examining allele mismatches between females and nestlings (see below). For groups with more than one adult female, we also compared genotypes of auxiliary females to those of the offspring.

We analyzed paternity in a two-step process. First, we used *CERVUS* to select the male from the population who, based on

Table 2
Variability at microsatellite loci used, based on analysis of 1996 samples (n = 287 to 290 individuals per locus)

Locus	No. alleles	Heterozygosity		Prob. of maternal exclusion	Prob. of paternal exclusion			Null allele frequency	Genotyping error rate
		Observed	Expected		r = .00	r = .25	r = .50		
Msp 4	10	0.635[a]	0.697	0.272	0.438	0.329	0.219	0.044	0.161
Msp 6	9	0.735	0.714	0.306	0.478	0.359	0.239	−0.017	0.000
Msp 10	14	0.875	0.858	0.555	0.716	0.537	0.358	−0.011	0.000
Mcy 3	14	0.826	0.847	0.546	0.709	0.532	0.355	0.011	0.016
Mcy 7	11	0.730[a]	0.832	0.512	0.682	0.512	0.341	0.061	0.086
Mcy 8	24	0.945	0.940	0.777	0.874	0.656	0.437	−0.004	0.017
Combined				0.989	0.999	0.984	0.909		

[a] Significantly different from expected; goodness-of-fit tests, df = 1, $p < .05$.

Results for other years were similar. Probability of maternal exclusion is the probability that a randomly selected adult will not match the nestling at a locus (when neither parent is known), and probability of paternal exclusion is the probability of excluding a randomly selected male as the sire, given the genotype of the mother and nestling (given for males of varying degrees of relatedness [r] to the true sire, after Double et al., 1997a). Null allele frequencies were estimated by *CERVUS* using the procedure of Summers and Amos (1997). Genotyping error rates were determined from comparisons between nestlings and their presumed mothers.

genetic evidence, had the highest likelihood of being the sire. *CERVUS* does this by calculating a likelihood score (the LOD score) for each male based on the offspring and maternal genotypes and taking into account scoring errors (e.g., due to null alleles); the male with the highest LOD score is selected as the most likely sire. *CERVUS* also uses a Monte Carlo simulation to determine the confidence (probability of a type I error) for each paternity assignment. This confidence measure is based on the parameter delta, which is the difference between the LOD for the most likely sire and the next most likely sire (Marshall et al., 1998). For these simulations we estimated the number of candidate males (58 to 93, depending on year) and proportion of males sampled (0.862 to 0.943) from field data, and we estimated genotyping error rates from mother/offspring comparisons.

Second, for each paternity assignment, we used a "total evidence" approach to determine whether we felt the *CERVUS* assignment was reasonable (see Prodöhl et al., 1998). In most cases we accepted the *CERVUS* assignment if the selected male had zero or one mismatch with the nestling, but we rejected the *CERVUS* assignment if the selected male showed two or more mismatches. In addition, we rejected the *CERVUS* assignment and assigned paternity to a lower-ranked male under three circumstances: (1) if both males had similar LOD scores but the lower-ranked male had fewer mismatches; (2) if both males had a single mismatch but the lower-ranked male's mismatch was consistent with the presence of a null allele (particularly at loci *Msp4* and *Mcy7*, see Results); and (3) if the males had the same low number of mismatches (0 or 1) and similar LOD scores, but independent evidence suggested that the lower-ranked male was a more likely sire. In this last case we considered whether either male was the social father, whether either male sired other young in the nest, or whether either male's mismatch was likely caused by a scoring error (e.g., mismatched alleles differed in size by only one repeat unit). These rules likely improved the accuracy of our assignments, particularly by reducing the influence of null alleles, but are unlikely to have affected our overall patterns, because we accepted the *CERVUS* male in the majority of cases and because exceptions typically occurred in cases where the delta value was low.

We examined intragroup relatedness in groups with auxiliary males, particularly in those groups in which the auxiliary appeared to sire young. Specifically, we compared the genotype of the auxiliary to that of the breeding pair to determine whether the auxiliary was likely to be the offspring of the breeding pair from an earlier breeding attempt. In these cases, the breeders were considered to be parents of the auxiliary if they could account for the auxiliary's alleles at all loci. If this was not the case, then one or both parents were excluded as the parents if they did not match the auxiliary at two or more loci (cases of a single mismatch were considered ambiguous). In addition, we estimated pairwise relatedness between female breeders and male auxiliaries within each group using the program SPAGeDi 1.0 (Hardy and Vekemans, 2002), using a "two-genes" relationship coefficient, r, based on Queller and Goodnight's (1989) formula.

Statistical analysis

All statistical analyses were conducted with Statview 5.0. Groups persisted across years, but composition often changed from one year to the next; therefore, we treated each group-year as an independent sample. For analyses examining factors associated with the frequency of extrapair young, we included only one brood per group per year to avoid problems of non-independence among data points. However, if group composition changed within a breeding season by replacement of the breeding male or female, we considered the resulting group to be a new group (five cases in three years). For analysis of patterns across years, we included only those groups for whom paternity analyses had been conducted. Each year, many groups failed to produce offspring or the offspring were depredated prior to sampling; thus, the number of groups sampled for paternity is lower than the number of groups studied. Additionally, although fieldwork was conducted during 1994, a severe drought throughout southern Australia that year dramatically affected our study population, and only two of more than 60 breeding groups produced offspring. Data from these two groups are not included in our analyses.

RESULTS

Analysis of maternity and genotyping errors

All six microsatellites were highly variable, with up to 25 alleles and high heterozygosities (Table 2). Consequently, the probability of excluding a false mother was high, ranging from 0.983 to 0.990 across years for all loci combined. We had a total of 450 mother-offspring pairs in which both individuals were genotyped at five or six microsatellite loci (98.9% of the offspring and 99.7% of the mothers were genotyped at six loci). Of these, 381 (84.7%) were cases with no mismatches

Table 3
Extrapair parentage and breeding group characteristics across years

Year	No. groups studied	Broods		Nestlings		Group characteristics		
		No. analyzed	Containing EPY (% ± 95% CI)	No. analyzed	EPY (% ± 95% CI)	Group size	No. auxiliaries	Sex ratio
1992	35	15	5 (33.3 ± 23.9)	34	8 (23.5 ± 14.3)	2.40	0.33	1.30
1993	42	21	13 (61.9 ± 20.8)	56	25 (44.6 ± 13.0)	3.14	0.76	1.41
1995	64	35	22 (62.9 ± 16.0)	85	34 (40.0 ± 10.4)	2.37	0.37	1.37
1996	69	39	22 (56.4 ± 15.6)	91	47 (51.6 ± 10.3)	2.77	0.62	1.46
1997	59	28	16 (57.1 ± 18.3)	70	31 (44.3 ± 11.6)	2.39	0.39	1.39
1998	50	21	10 (47.6 ± 21.4)	50	18 (36.0 ± 13.3)	2.43	0.24	1.12
Total	319	159	88 (55.4 ± 7.7)	386	163 (42.2 ± 4.9)	2.59	0.47	1.36

Table includes only the first brood from each group; second broods were excluded unless the composition of the group (identity of breeding male or female) changed between the first and second brood. If the composition changed, the brood from the 'new group' was included (five cases in three years). EPY refers to nestlings sired by extrapair males. Group sex ratio is number of adult males divided by number of adult females. Confidence intervals (95% CI) were calculated assuming a binomial distribution. Group characteristics are for all groups with offspring genotyped.

between the mother and nestling, and 69 (15.3%) were cases that showed one or more loci mismatching.

Mismatches between a nestling and its presumed mother could be caused by misassigned maternity (e.g., brood parasitism), mutation, or scoring errors. Our data indicate that most or all of the mismatches were caused by mutations and scoring errors rather than by misassigned maternity. First, misassigned maternity would likely result in the mother and nestling mismatching at several loci, but in our analyses 66 (95.7%) were cases with a single mismatch, and only 3 (4.3%) were cases in which the presumed mother and nestling mismatched at multiple loci (2 loci in all cases). Second, scoring errors can be caused by null alleles, and nearly half (35 of 72) of our mismatches showed a pattern consistent with the presence of a null allele (female and offspring appear homozygous, but for different alleles). Virtually all of these apparent nulls (33 of 35 mismatches) occurred at two loci: *Msp4* (10 of 22 mismatches at this locus) and *Mcy7* (23 of 27). Null alleles appeared to be particularly prevalent at the latter locus; observed heterozygosity was significantly lower than expected ($p < .05$ after Bonferonni adjustment) in 4 of 6 years, and the estimated null allele frequency ranged from 0.03 to 0.07 across years. Locus *Msp4* also showed a relatively high estimated frequency of null alleles in some years (up to 0.06) but did not show a significant deficiency of heterozygotes in any year after adjustment for multiple tests. None of the other loci showed significant deviation from Hardy-Weinberg expectations.

If we accept cases of a single mismatch between a mother and offspring as cases of scoring error rather than misassigned maternity, then we have only 3 cases (0.7% of 450 nestlings) that represent possible cases of brood parasitism (2 or more mismatches). This is likely an overestimate of the frequency of brood parasitism, as double scoring errors cannot be ruled out in these three cases. Thus, we conclude that presumed mothers are the biological mothers of their nestlings in the vast majority of, if not all, cases.

General patterns of paternity and the role of auxiliaries

We excluded the following nestlings from paternity analyses: those that had fewer than 5 loci scored ($n = 2$), those whose presumed mothers had not been genotyped ($n = 6$), and those that showed more than a single mismatch with their presumed mothers ($n = 3$). This left a total of 447 nestlings for paternity analyses.

Using our parameter settings, *CERVUS* assigned a sire to 446 of these 447 nestlings: 277 were assigned with 95% confidence and 169 were assigned with 80% confidence. Using our rules for accepting or rejecting the *CERVUS* assignments (see Methods), we accepted 96.4% of the high confidence assignments and 60.4% of the low confidence assignments. In some cases where we rejected the *CERVUS* assignment, the nestling was assigned to another male using our decision rules, whereas in other cases the nestling was not assigned to any male. In total, sires were assigned to 430 (96.2%) of the nestlings analyzed.

Our paternity assignments indicated a high level of extrapair paternity in this population. Across the six years, an average of 55.3% of all broods analyzed contained offspring sired by males other than the dominant male in the social group, and 42% of all offspring were the result of such matings (Table 3). The distribution of extrapair young among broods was nonrandom (Figure 1), with more broods containing zero or many extrapair young per brood than would be expected by chance. In some cases (36 of 183 broods) an entire brood was sired by a single extrapair male. Altogether, mixed paternity (more than one sire) occurred in 66 (36%) of 183 broods.

The frequency of extrapair young varied across years (Table 3). Examining mean values each year, the proportion of young that were sired by extrapair males was significantly and positively correlated with the mean number of auxiliaries per group in each year (Figure 2). We examined the role of auxiliaries more directly by combining the data across years. The proportion of a female's offspring that were sired by extrapair males varied significantly with the number of auxiliaries in the group (ANOVA, $F_{3,155} = 3.761$, $p = .012$). Specifically, groups with two or more auxiliaries had a higher proportion of extrapair offspring than did groups with zero or one auxiliary (Figure 3). Similarly, the average size of groups containing males that were completely cuckolded (i.e., sired no within-pair offspring; group size = 3.05 ± 1.11, $n = 40$) was significantly larger than the average size of groups containing males that sired at least some of the progeny in their brood (2.42 ± 0.67, $n = 118$, $t = -4.260$, df = 157, $p < .001$).

Some extrapair young were sired by auxiliary males from the same group (see below), and the frequency of these young was related to group size (Figure 3; ANOVA, $F_{2,55} = 3.672$, $p = .032$; LSD post hoc tests indicated that differences were between groups with two helpers vs. groups with zero or one helper). When young sired by group auxiliaries were excluded, there was not a relationship between the proportion of offspring

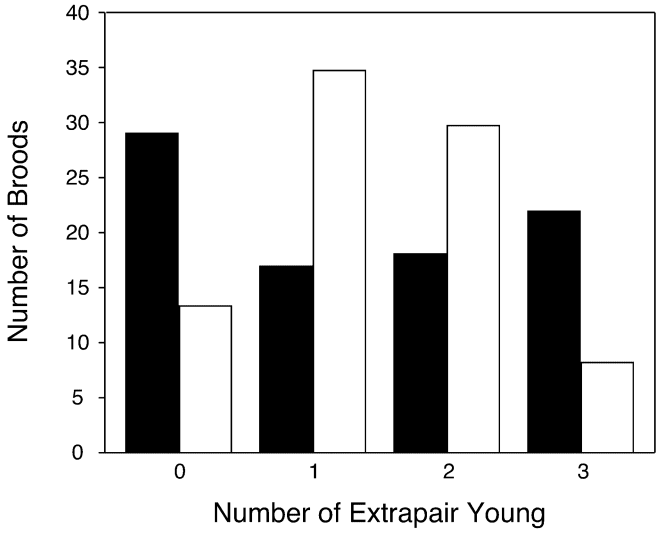

Figure 1
Number of extrapair young per brood (dark bars) and expected number per brood (white bars) for broods of three nestlings (the modal brood size, $n = 86$ broods). Expected values calculated from the multivariate hypergeometric distribution (Neuhäuser et al., 2001) using the number of extrapair young seen in the included broods (119 of 258 offspring). The observed and expected distributions differ significantly ($\chi^2 = 55.11$, df = 3, $p < .0001$).

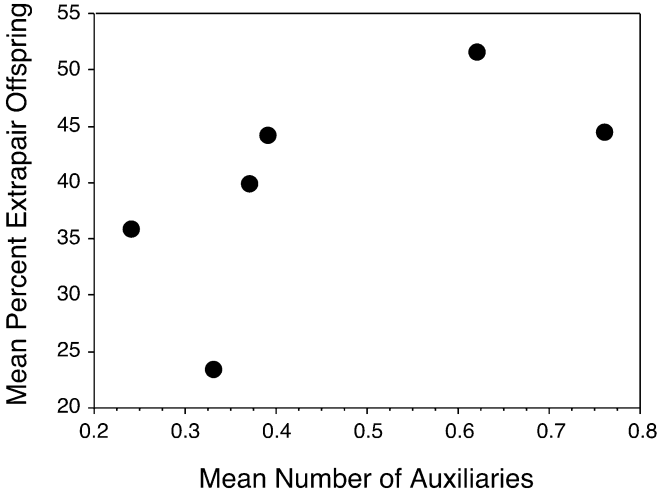

Figure 2
Proportion of sampled nestlings that were sired by extrapair males versus the mean number of males per group. Each point represents averages for one year. Number of auxiliaries per group was correlated significantly with frequency of EPF across years (Spearman rank correlation, analysis restricted to first broods from groups that had offspring genotyped, $\rho = 0.886$, $p = .048$).

sired by extragroup males and the number of auxiliaries in the group (ANOVA, $F_{3,155} = 1.096$, $p = .353$), though the trend was in the same direction as discussed above (Figure 3).

The number of auxiliaries did not appear to affect the likelihood that a breeding male would sire extrapair young. The average size of groups containing males that sired extrapair young (2.56 ± 0.94, $n = 39$) did not differ significantly from the average size of groups containing males who did not sire extrapair young (2.59 ± 0.82, $n = 120$, $t = 0.177$, df = 157, $p = .861$).

Identities of extrapair sires and paternity by auxiliaries

Most extrapair young were sired by dominant breeding males from outside the group (Table 4), and these were usually neighboring males (Pruett-Jones et al., in preparation). Nevertheless, 10.0% of all offspring analyzed, or 24.6% of all extrapair young, were sired by auxiliary males (Table 4). Approximately half of these offspring were sired by auxiliaries from other groups and half were sired by auxiliaries from within their own group.

There were a total of 12 cases in which an auxiliary male sired young in his own group ($n = 18$ young total, in only one case was the same auxiliary/female pair involved in two different years). In eight of these cases, genetic data indicated that the auxiliary was not the offspring of the breeding female, and in six of these cases our behavioral and demographic data confirmed that the breeding female had joined the group after the birth of the auxiliary. In two additional cases the genetic data were ambiguous, and in two cases the auxiliary appeared to be the son of the breeding female. Being as conservative as possible, auxiliary sons sired young with their mothers in a maximum of four cases (0.9% of all offspring analyzed).

If incest avoidance limits reproduction by auxiliaries within their own group, the likelihood that an auxiliary male sires offspring in his own group should increase as relatedness between the auxiliary and the breeding female decreases, which is likely to occur when a breeding female is replaced. We tested this prediction by tabulating the number of broods in which the auxiliary did or did not sire offspring, limiting our analysis to broods in which we could infer whether or not the auxiliary was a son of the breeding female (based on banding records and genetic analysis, see Methods). This analysis indicated that an auxiliary male was more likely to sire young within the group when he was not a son of the breeding female (8 of 20 cases) than in situations in which he was the son of the breeding female (2 of 21 cases; df = 1, Fisher's exact $p = .033$). By way of contrast, relatedness between the auxiliary and breeding female did not affect the probability that a brood would contain young sired by a male from outside the group (df = 1, Fisher's exact $p = .734$). Thus, our results indicate that an auxiliary male is unlikely to sire young in his own group if the breeding female is his mother, but he is much more likely to do so if he is unrelated to the breeding female.

Compared to females with a single auxiliary, females with multiple auxiliaries may have a higher probability of being unrelated to at least one of those auxiliaries, and this may contribute to the relationship between group size and extrapair paternity (Figure 3). However, we found no relationship between the number of auxiliaries and the average relatedness between the female breeder and auxiliaries (ANOVA, $F_{2,50} = 0.572$, $p = .568$). We also examined whether the number of auxiliaries affected the probability that the group would contain at least one auxiliary with low relatedness to the breeding female (i.e., below the median relatedness between females and their auxiliaries, $r = .302$). We found no such relationship: seven of 11 groups (63.6%) with multiple auxiliaries had at least one auxiliary of low relatedness to the breeding female, whereas the auxiliary showed low relatedness to the female in 22 of 41 groups (53.7%) with a single auxiliary (df = 1, Fisher's exact $p = .735$).

Our results suggest that auxiliary reproduction may be affected by relatedness between the auxiliary and breeding males. Of the 12 cases in which an auxiliary male sired young within the group, the auxiliary was unrelated to the breeding male in seven cases and appeared to be related in two cases (three cases were ambiguous). Given the overall rates of extrapair fertilizations (Table 3), we would expect the auxiliary

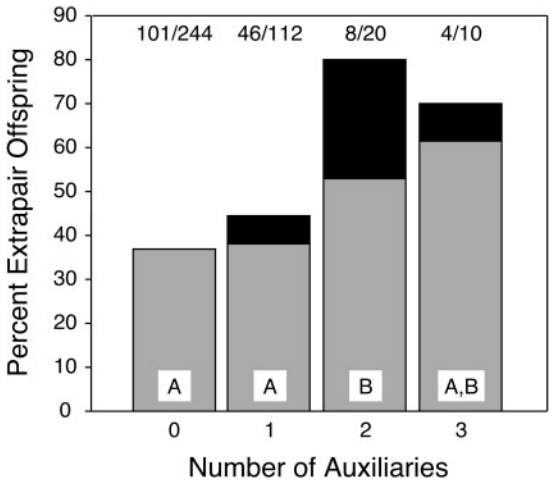

Figure 3
Percentage of offspring that were sired by extrapair males as a function of number of auxiliaries. Bars with different letters (A or B) differ significantly from each other (ANOVA post hoc LSD tests, $p \leq .05$). Numbers above bars are number of broods analyzed over number of offspring analyzed. The grey portion of each bar indicates young sired by extragroup males, whereas the black portion indicates young sired by group auxiliaries. Differences among bars are not significant if young sired by group auxiliaries are excluded (see text).

and breeding males to be unrelated in approximately three to four of nine cases. Although the sample size is too small for proper statistical analysis, these results suggest that an auxiliary male is more likely to gain sirings within the group if he is unrelated to the breeding male.

DISCUSSION

General patterns and the effects of group size

Detailed studies of splendid fairy-wrens (Brooker et al., 1990; Rowley and Russell, 1990a) were among the first to document that reproductive promiscuity occurs at high levels in some socially monogamous birds, including cooperative breeders. Our study confirms this pattern for another population of the same species, as we found that over 40% of the offspring analyzed were sired by extrapair males. Indeed, this pattern fits the general pattern seen within the avian family Maluridae—reproductive promiscuity appears to be common in all species studied to date (Brooker et al., 1990; Dunn and Cockburn, 1998; Karubian, 2002; Mulder et al., 1994), and similar levels of promiscuity are suspected in species of *Malurus* that have not been studied with genetic methods (Rowley and Russell, 1997).

Our data suggest that rates of reproductive promiscuity in the eastern subspecies of splendid fairy-wrens are lower than those of the western subspecies, *M. s. splendens*, for which a previous study found that over two-thirds of all offspring were sired by extrapair males (Brooker et al., 1990; Rowley and Russell, 1990a). This difference may be due to the molecular markers used in the two studies (allozymes vs. microsatellites) or possibly to ecological differences between the two study populations. In our population, density of breeding pairs, group size, clutch size, reproductive success, and annual survivorship are all lower than in the population studied by Rowley and associates (Rowley and Russell, 1997; Van Bael and Pruett-Jones, 2000; Pruett-Jones S, unpublished data). Of these factors, density of breeding pairs (Griffith et al., 2002; Møller and Ninni, 1998; Richardson and Burke, 2001; Westneat and Sherman, 1997) and average group size (this study) have both been shown to affect rates of extrapair fertilization, and so these may account for the differences seen between eastern and western races of splendid fairy-wrens.

Table 4
Identity of sires for all offspring for whom sire was identified (n = 430), regardless of brood

Identity of sire	No. of cases	% Total	% Extrapair young
Dominant male in same group	255	59.3	—
Auxiliary male in same group	18	4.2	10.3
Dominant male in another group	132	30.7	75.4
Auxiliary male in another group	25	5.8	14.3

Offspring that were genotyped but for whom sire was not identified are excluded (n = 17).

Individual reproductive decisions may be influenced by a number of factors, particularly in cooperative systems with complex groups. For example, Mulder et al. (1994) suggested that females in groups with auxiliaries may be "liberated" to pursue extrapair fertilizations, because auxiliaries would help compensate for reduced male parental care should it occur (the "female emancipation hypothesis"). In support of this hypothesis, Mulder et al. (1994) showed that in the superb fairy-wren (*M. cyaneus*) extrapair young were more common in groups with auxiliaries than in groups without auxiliaries. We found a very similar pattern in this study (Figure 3), supporting the female emancipation hypothesis. In our study, however, groups with only a single male auxiliary were no more likely to have extrapair young than those without auxiliaries; the effect of auxiliaries only appeared in groups with at least two auxiliaries. This may be because, under the relatively harsh conditions experienced by our study population, assistance by two or more auxiliaries is necessary to compensate the female for any possible reduction in paternal care by the dominant male.

Although the association between frequency of extrapair young and group size supports the female emancipation hypothesis, other explanations are possible. For example, territory quality may affect both reproductive success (and hence number of auxiliaries) and the propensity for females to pursue EPF (Gowaty, 1996). Alternatively, females with auxiliaries may have reduced feeding loads, and hence more time to pursue EPF (although this does not appear to be the case in superb fairy-wrens; Dunn and Cockburn, 1996), or auxiliaries may interfere with courtship of the breeding pair. Finally, the relationship may reflect sirings by group auxiliaries if females with more auxiliaries have a higher probability of being unrelated to at least some of those auxiliaries. In this study we found that most sirings by group auxiliaries were in larger groups (Figure 3), and the relationship between number of auxiliaries and proportion of extrapair young was not significant if sirings by group auxiliaries were excluded. However, there was still a trend for larger groups to have more young sired by extragroup males (Figure 3) and, more importantly, we did not find that females with more auxiliaries had a higher probability of being unrelated to those auxiliaries. In sum, although a relationship between the number of auxiliaries and EPF rates has been found in two different species of fairy-wren, the underlying cause of this relationship remains unclear; detailed behavioral observations, and possibly experimental approaches, will be necessary to evaluate these alternatives.

Whatever its cause, the association between EPF rates and group size raises the question as to why a breeding male would tolerate auxiliaries on his territory—if the presence of auxiliaries is likely to reduce the dominant male's genetic contribution to the brood, why does he not drive those auxiliaries away? One possibility is that auxiliaries increase the breeding male's reproductive success enough to compensate for reduced paternity within the brood. This possibility may be unlikely for splendid fairy-wrens, as auxiliaries appeared to increase the reproductive success of breeding adults only moderately in a western population (Rowley and Russell, 1990b). Alternatively, auxiliaries may liberate the breeding male from parental duties and allow him to pursue EPF himself (Green et al., 1995), such that increased extrapair success compensates for reduced paternity within his own brood. Our results do not support this hypothesis, as group size did not differ between males who did and did not sire extrapair young. A third alternative is that breeding males do not drive auxiliaries from the territory because this would reduce the breeding male's inclusive fitness if those sons have few breeding opportunities (Pruett-Jones and Lewis, 1990). This and other possible explanations require further testing; at present it is unclear why breeding males tolerate auxiliaries on their territories.

Reproduction by auxiliaries and conflicts within groups

Few studies of cooperatively breeding birds have assigned extrapair and extragroup paternity to specific males (Cockburn et al., 2003; Double and Cockburn, 2003; Richardson et al., 2001), and so the extent to which auxiliary males sire offspring has not been clear in most systems. In this study we found that approximately 25% of all extrapair offspring, or 10% of all offspring analyzed, were sired by auxiliary males. Thus, auxiliary males are able to obtain some direct fitness while assisting a breeding pair. Auxiliary males in fairy-wren species are likely prevented from independent breeding by limited breeding opportunities (Pruett-Jones and Lewis, 1990), and these small fitness gains may help tip the cost/benefit balance toward remaining on the natal territory as an auxiliary rather than dispersing to search for (rare) breeding opportunities. Indeed, given that the indirect fitness benefits of helping are likely small (Rowley and Russell, 1990b), direct fitness benefits such as EPF and increased survival may be the primary benefits of remaining on the natal territory as an auxiliary.

Our analyses indicated that auxiliary males sired young within their own breeding groups as well as in other breeding groups. Within-group reproduction by auxiliaries is relatively common in some cooperatively or communally breeding species (Baglione et al., 2002; Li and Brown, 2000; Whittingham et al., 1997), but in most of these cases auxiliaries are males who join the group and are unrelated to the breeding female (see Lundy, 1998). In most cases where offspring remain with the natal group to help their parents, male auxiliaries rarely sire offspring within their group (e.g., Dickinson and Akre, 1998; Haig et al., 1994; Hatchwell et al., 2002; Quinn et al., 1999), at least so long as the group remains intact. Splendid fairy-wrens appear to fit this general pattern of incest avoidance.

Emlen (1995, 1997) predicted that reproductive conflicts of interest should arise in such systems whenever a new female joins an established group with breeding and auxiliary males, because in this situation all group males would be unrelated to the new breeding female. Our data confirm this prediction for splendid fairy-wrens, as auxiliaries sired within-group offspring in approximately one-half of the cases in which a new female joined the group (see also Cockburn et al., 2003; Piper and Slater, 1993; Rabenold et al., 1990). Interestingly, our data also suggest (albeit with small sample size) that auxiliary males are also more likely to sire young within their group when they are unrelated to the breeding male, a pattern that is consistent with the hypothesis that breeding males must concede some reproduction to unrelated auxiliaries to retain their help (Emlen, 1997). With frequent EPF, auxiliary males will be frequently unrelated to the breeding male they are assisting, and these circumstances would tip the selective balance more strongly toward within-group paternity by auxiliary males. Thus, high levels of EPF lead to a situation which favors within-group sirings by auxiliary males and hence increase the reproductive conflict between the breeding and auxiliary males.

We wish to thank the numerous field and lab assistants that helped us during this work, the South Australian government for permission to carry out this research, and Stephanie Williams, Joe Stelman, the Brookfield Conservation Park Friends Group, and Mary Ashley for logistical and other assistance. Comments of Andrew Bourke and two anonymous referees improved the manuscript substantially. This research was conducted with proper authorization and using protocols approved by the University of Chicago Institutional Animal Care and Use Committee. Financial support was provided by the Chicago Zoological Society, the M. A. Ingram Trust, the Norman Wettenhall Foundation, and by grants from the National Science Foundation to S.P.-J. and M.S.W.

REFERENCES

Baglione V, Marcos JM, Canestrari D, Ekman J, 2002. Direct fitness benefits of group living in a complex cooperative society of carrion crows, *Corvus corone corone*. Anim Behav 64:887–893.

Brooker MG, Rowley I, Adams M, Baverstock PR, 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? Behav Ecol Sociobiol 26:191–199.

Bruce JP, Quinn JS, Sloane SA, White BN, 1996. DNA fingerprinting reveals monogamy in the bushtit, a cooperatively breeding species. Auk 113:511–516.

Clutton-Brock, T. H. 2002. Breeding together: Kin selection and mutualism in cooperative vertebrates. Science 296:69–72.

Cockburn A, 2003. Mating systems and sexual conflict in cooperatively breeding birds. In: Cooperative Breeding in Birds: Recent Research and New Theory (Koenig WD, Dickinson JL, eds). Cambridge: Cambridge University Press; in press.

Cockburn A, Osmond HL, Mulder RA, Green DJ, Double MC, 2003. Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren (*Malurus cyaneus*). J Anim Ecol 72:189–202.

Dickinson JL, Akre JJ, 1998. Extrapair paternity, inclusive fitness, and within-group benefits of helping in western bluebirds. Molec Ecol 7: 95–105.

Dickinson JL, Haydock H, Koenig W, Stanback M, Pitelka F, 1995. Genetic monogamy in single-male groups of acorn woodpeckers, Melanerpes formicivorus. Molec Ecol 4:765–769.

Double MC, Cockburn A, 2003. Subordinate superb fairy-wrens (*Malurus cyaneus*) parasitize the reproductive success of attractive dominant males. Proc R Soc Lond B 270:379–384.

Double MC, Cockburn A, Barry SC, Smouse PE, 1997a. Exclusion probabilities for single-locus paternity analysis when related males compete for matings. Molec Ecol 6:1155–1166.

Double MC, Dawson D, Burke T, Cockburn A, 1997b. Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. Molec Ecol 6:691–693.

Dunn, P. O., A. Cockburn. 1996. Evolution of male parental care in a bird with almost complete cuckoldry. Evolution 50:2542–2548.

Dunn PO, Cockburn A, 1998. Costs and benefits of extragroup paternity in Superb fairy-wrens. In: Avian Reproductive Tactics: Female and Male Perspectives, Ornithological Monographs No. 49 (Parker PG, Burley NT, eds). Washington, D. C.: American Ornithologists' Union; 147–161.

Dunn PO, Cockburn A, Mulder RA, 1995. Fairy-wren helpers often care for young to which they are unrelated. Proc R Soc Lond B 259: 339–343.

Emlen ST, 1991. Evolution of cooperative breeding in birds and mammals. In: Behavioral Ecology: An Evolutionary Approach, 3rd ed (Krebs JR, Davies NB, eds). Oxford: Blackwell; 301–337.

Emlen ST, 1995. An evolutionary theory of the family. Proc Natl Acad Sci USA 92:8092–8099.

Emlen ST, 1997. Predicting family dynamics in social vertebrates. In: Behavioural Ecology: An Evolutionary Approach, 4th ed (Krebs JR, Davies NB, eds). Oxford: Blackwell Science; 228–253.

Emlen ST, Wrege PW, Webster MS, 1998. Cuckoldry as a cost of polyandry in the sex-role reversed wattled jacana. Proc R Soc Lond B 265:2359–2364.

Gowaty PA, 1996. Battles of the sexes and origins of monogamy. In: Partnerships in Birds: The Study of Monogamy (Black JM, ed). Oxford: Oxford University Press; 21–52.

Green DJ, Cockburn A, Hall ML, Osmond H, Dunn PO, 1995. Increased opportunities for cuckoldry may be why dominant male fairy-wrens tolerate helpers. Proc R Soc Lond B 262:297–303.

Griffin, AS, SA West. 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 302: 634–636.

Griffith SC, Owens IPF, Thuman KA, 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molec Ecol 11:2195–2211.

Haig SM, Walters JR, Plissner JH, 1994. Genetic evidence for monogamy in the cooperatively breeding red-cockaded woodpecker. Behav Ecol Sociobiol 34:295–303.

Hammond RL, Saccheri IJ, Ciofi C, Coote T, Funk SM, McMillan WO, Bayes MK, Taylor E, Bruford MW, 1998. Isolation of microsatellite markers in animals. In: Molecular Tools for Screening Biodiversity: Plants and Animals (Karp A, Isaac PG, Ingram DS, eds). London: Chapman and Hall; 279–285.

Hardy OJ, Vekemans X, 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620.

Hatchwell BJ, Ross DJ, Chaline N, Fowlie MK, Burke T, 2002. Parentage in the cooperative breeding system of long-tailed tits, *Aegithalos caudatus*. Anim Behav 64:55–63.

Haydock J, Parker PG, Rabenold KN, 1996. Extrapair paternity uncommon in the cooperativley breeding bicolored wren. Behav Ecol Sociobiol 38:1–16.

Jamieson A, 1994. The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigress and (2) distinguishing full-sib pair members. Animal Genetics 25, supplement 1:37–44.

Jamieson IG, Quinn JS, Rose PA, White BN, 1994. Shared paternity among non-relatives is a result of an egalitarian mating system in a communally breeding bird, the pukeko. Proc R Soc Lond B 257: 271.

Jennions, M. D., D. W. Macdonald. 1994. Cooperative breeding in mammals. Trends in Ecology and Evolution 9:89–93.

Joste N, Ligon JD, Stacey PB, 1985. Shared paternity in the acorn woodpecker. Behav Ecol Sociobiol 17:39–41.

Karubian J, 2002. Costs and benefits of variable breeding plumage in the red-backed fairy-wren. Evolution 56:1673–1682.

Lessios HA, 1992. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Marine Biol 112:517–523.

Li S-H, Brown JL, 2000. High frequency of extrapair fertilization in a plural breeding bird, the Mexican jay, revealed by DNA microsatellites. Anim Behav 60:867–877.

Lundy KJ, Parker PG, Zahavi A, 1998. Reproduction by subordinates in cooperatively breeding Arabian babblers is uncommon but predictable. Behav Ecol Sociobiol 43:173–180.

Magrath RD, Whittingham LA, 1997. Subordinate males are more likely to help if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. Behav Ecol Sociobiol 41:185–192.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molec Ecol 7:639–655.

Møller AP, Ninni P, 1998. Sperm competition and sexual selection: A meta-analysis of paternity studies in birds. Behav Ecol Sociobiol 43: 345–358.

Mulder RA, Dunn PO, Cockburn A, Lazenby-Cohen KA, Howell MJ, 1994. Helpers liberate female fairy-wrens from constraints on extrapair mate choice. Proc R Soc Lond B 255:223–229.

Nei M, 1987. Molecular Evolutionary Genetics. New York: Columbia University Press.

Neuhäuser M, Forstmeier W, Bretz F, 2001. The distribution of extrapair young within and among broods – a technique to calculate deviations from randomness. J Avian Biol 32:271–274.

Pemberton JM, Slate J, Bancroft DR, Barrett JA, 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. Molec Ecol 4:249–252.

Piper WH, Slater G, 1993. Polyandry and incest avioidance in the cooperative stripe-backed wren. Behaviour 124:227–247.

Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Thompson EA, Avise JC, 1998. Genetic maternity and paternity in a local population of armadillos assessed by microsatellite DNA markers and field data. Am Nat 151:7–19.

Pruett-Jones SG, Lewis MJ, 1990. Sex ratio and habitat limitation promote delayed dispersal in superb fairy-wrens. Nature 348:541–542.

Queller DC, Goodnight KF, 1989. Estimating relatedness using genetic markers. Evolution 43,258–275.

Quinn JS, Woolfenden GE, Fitzpatrick JW, White BN, 1999. Multilocus DNA fingerprinting supports genetic monogamy in Florida scrub-jays. Behav Ecol Sociobiol 45:1–10.

Rabenold PP, Rabenold KN, Piper WH, Haydock J, Zack SW, 1990. Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. Nature 348:538–540.

Richardson DS, Burke T, 2001. Extrapair paternity and variance in reproductive success related to breeding density in Bullock's orioles. Anim Behav 62:519–525.

Richardson DS, Burke T, Komdeur J, 2002. Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. Evolution 56:2313–2321.

Richardson DS, Jury FL, Blaakmeer K, Komdeur J, Burke T, 2001. Parentage assignment and extragroup paternity in a cooperative breeder: The Seychelles warbler (*Acrocephalus sechellensis*). Molec Ecol 10:2263–2273.

Richardson DS, Komdeur J, Burke T, 2003. Altruism and infidelity among warblers. Nature 422:580.

Rowley I, Russell E, 1990a. "Philandering" – a mixed mating strategy in the splendid fairy-wren *Malurus splendens*. Behav Ecol Sociobiol 27:431–437.

Rowley I, Russell E, 1990b. Spendid fairy-wrens: demonstrating the importance of longevity. In: Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior (Stacey PB, Koenig WD, eds). Cambridge: Cambridge University Press; 1–30.

Rowley I, Russell E, 1997. Fairy-wrens and Grasswrens, Maluridae. Oxford: Oxford University Press.

Rowley I, Russell E, Payne RB, Payne LL, 1989. Plural breeding in the splendid fairy-wren, *Malurus splendens* (Aves: Maluridae), a cooperative breeder. Ethology 83:229–247.

Summers K, Amos W, 1997. Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. Behav Ecol 8:260–267.

Tibbetts E, Pruett-Jones S, 1999. Habitat and nest-site partitioning in splendid and variegated fairy-wrens (Aves: Maluridae). Australian J Zool 47:317–326.

Van Bael S, Pruett-Jones S, 2000. Breeding biology and social behaviour of the eastern race of the splendid fairy-wren *Malurus splendens melanotus*. Emu 100:95–108.

Westneat DF, Sherman PW, 1997. Density and extrapair fertilizations in birds: a comparative analysis. Behav Ecol Sociobiol 41:205–215.

Whittingham LA, Dunn PO, Magrath RD, 1997. Relatedness, polyandry and extragroup paternity in the cooperatively-breeding white-browed scrubwren (*Sericornis frontalis*). Behav Ecol Sociobiol 40: 261–270.

Wrege PH, Emlen ST, 1987. Biochemical determination of parental uncertainty in white-fronted bee-eaters. Behav Ecol Sociobiol 20: 153–160.