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DIRECT BENEFITS AND THE EVOLUTION OF FEMALE-BIASED COOPERATIVE BREEDING IN SEYCHELLES WARBLERS

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Abstract.—Inclusive fitness benefits have been suggested to be a major selective force behind the evolution of cooperative breeding. We investigated the fitness benefits selecting for cooperative breeding in the Seychelles warbler, *Acrocephalus sechellensis*. A microsatellite-based genotyping method was used to determine the relatedness of subordinates to group offspring in an isolated population of Seychelles warblers. The indirect and direct breeding benefits accruing to individual subordinates were then calculated for every successful breeding event over a three-year period. We show that female subordinates frequently gained parentage and that this, combined with high levels of extragroup paternity, resulted in low levels of relatedness between subordinates and nondescendent offspring within a territory. Direct breeding benefits were found to be significantly higher than indirect kin benefits for both female and male subordinates. As predicted, female subordinates gained significantly more direct breeding opportunities and therefore higher inclusive fitness benefits by being a subordinate within a group than did males. This may explain why most subordinates in the Seychelles warbler are female.

Key words.—*Acrocephalus* warblers, cooperative breeding, direct benefits, indirect benefits, kin selection, microsatellite markers, relatedness.

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In vertebrate cooperative breeding systems adult individuals other than the breeding pair assist in rearing offspring from a single breeding attempt. In these systems some individuals care for young that are not their own genetic offspring (Brown 1987). This provides an apparent paradox for current evolutionary thinking and, consequently, many studies have investigated the factors that favor the evolution and maintenance of cooperative breeding (reviewed in Stacey and Koenig 1990; Koenig et al. 1992; Jennions and Macdonald 1994; Emlen 1995; Cockburn 1998; Arnold and Owens 1998; Hatchwell and Komdeur 2000). In most cooperatively breeding vertebrates, individuals delay dispersal and help their parents raise subsequent offspring. Consequently, several authors have suggested that indirect fitness benefits (kin selection, Hamilton 1964) are a driving force behind the evolution of cooperative breeding (Emlen and Wrege 1989; Mumme et al. 1989; Mumme 1992; Koenig et al. 1992, Emlen 1997). However, there are reports of cooperative systems containing unrelated subordinates (e.g., Reyer 1980; Sherley 1990; Whittingham et al. 1997; reviewed in Cockburn 1998) and many studies have found no correlation between helping effort and relatedness (Rabenold 1985; Zahavi 1990, Du Plessis 1993; Dunn et al. 1995; Magrath and Whittingham 1997; Clutton-Brock et al. 2000). These studies have led to the suggestion that the role of indirect benefits in cooperative systems has been overstated (Cockburn 1998), thus rekindling the earlier debate (see Reyer 1980 and references therein) about the relative importance of indirect benefits and direct benefits. Recent studies have fueled this debate with evidence for either direct or indirect benefits in different systems (Russell and Hatchwell 2001; Cant and Field 2001; Clutton-Brock et al. 2001).

One factor that complicates the study of benefits within cooperative breeding systems is that different members of the same group may incur different costs and benefits from helping (reviewed in Cockburn 1998; Heinsohn and Legge 1999). Studies on birds indicate that benefits differ between the sexes (reviewed in Cockburn 1998). Therefore, any study focusing on the benefits of cooperative breeding in systems with subordinates of both sexes should analyze the sexes separately; this might in turn help to explain the skewed sex ratios of subordinates often seen in cooperative species (West and Sheldon 2002).

Until recently, accurately determining parentage and relatedness within complex cooperative breeding systems was fraught with problems (e.g., McRae and Amos 1999; but see Parker et al. 1999). Consequently, accurately measuring the benefits accruing to subordinates was impossible and this may, in part, be responsible for the lack of a resolution to the question of which benefits drive cooperative breeding. The advent of powerful molecular genotyping techniques (Burke 1989; Queller 1993) means that we are now able to quantify the direct and indirect benefits accruing to subordinates within cooperatively breeding species.

The Seychelles warbler (*Acrocephalus sechellensis*) is a passerine bird endemic to the Seychelles Islands in the western Indian Ocean. Although warblers can breed independently in their first year, a lack of suitable independent breeding opportunities drives some individuals into becoming subordinates within their natal territory (Komdeur 1992). The majority of these subordinates are female (88%, $n = 217$; Komdeur 1996a).

Studies have shown that subordinate Seychelles warblers gain some direct benefits through cooperative breeding, that is, the acquisition of parental experience (Komdeur 1996b).

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Komdeur (1994a) also showed that indirect benefits are gained as subordinates help raise dependent young, to whom they appear to be related, and this behavior improves the reproductive success of the breeding pair. However, this study included the effect of all alloparents (subordinates and cobreeders combined) on the reproductive success of the breeding pair. Furthermore, the suggestion that helping is just a neutral behavioral response of adults to the presence of begging young (Jamieson 1991) can be actively rejected for the Seychelles warbler as subordinates help preferentially at nests where they are related to the primary birds (Komdeur 1994a).

A recent analysis of parentage in the Seychelles warbler shows that joint nesting occurs, with 44% of subordinate females producing offspring and 40% of young resulting from extragroup paternity (Richardson et al. 2001). These patterns of parentage will affect the benefits gained by subordinates. Direct benefits will be higher than previously predicted, with subordinates sharing reproduction, while indirect fitness benefits will be substantially less than suggested for two reasons: First, the increase in reproductive success seen on territories containing subordinates may be mainly the result of direct breeding by subordinate females. Second, the high frequency of extragroup paternity and shared reproduction will reduce the average coefficient of relatedness (r) between subordinates and nondescendent offspring and, therefore, reduce any indirect benefits.

Our study uses parentage analysis and a coefficient of relatedness, based on microsatellite markers, to determine, in terms of genetic equivalents, the exact direct breeding and indirect benefits for each subordinate within a population of the Seychelles warbler. Specifically we aimed to: (1) determine the different benefits gained by the male and female subordinates, and (2) assess the relative importance of direct versus indirect benefits in the evolution and maintenance of cooperative breeding in the Seychelles warbler. We predict that females will gain greater direct benefits than males through joint nesting and that this can explain why most subordinates are female in this species.

Materials and Methods

Study Population and Data Collection

The Cousin Island population of Seychelles warblers has been monitored intensively since December 1985. During this time nearly all birds within the population have been individually color-ringed (using a unique combination of three UV-resistant color rings and a British Trust for Ornithology metal ring) and monitored throughout breeding attempts. Therefore, the reproductive history, status, and putative pedigree of the majority of birds is known.

The present study was based on the 300–340 adult warblers observed per breeding season between 1997 and 1999. In the breeding seasons (June–September and December–February) each territory was checked for nesting activity at least once every two weeks by following the resident female for 30 min (Komdeur 1992). The Seychelles warbler produces one clutch per season and this normally consists of just one egg, but about 20% of nests contain two or three eggs (Komdeur 1991; Richardson et al. 2001). Activity by resident birds was observed at active nests for a minimum of 10 min every three to four days during nest building and incubation (14–16 days incubation period) and after hatching until fledging (18–20 days nestling period).

The status of all birds was based upon field observations from the given field season combined with the available long-term demographic data. For example, a single male and female initiated breeding on a territory and then gained subordinates in subsequent years, as old offspring remained resident within the territory. The ''primary'' male and female were defined as the dominant, pair-bonded male and female in the territory, while the term ''subordinate'' included all other birds (> eight months old) resident in the territory. Subordinates were split into two categories: subordinate parents and subordinate nonparents.

During the study almost all birds (approximately 96%) were color-ringed and blood-sampled. Blood samples (ca 15 μl) were collected by brachial venipuncture, diluted in 800 μl of 100% ethanol in a screw-cap microfuge tube and stored at room temperature. Molecular sexing using the polymerase chain reaction (PCR) method devised by Griffiths et al. (1998) was used to confirm the sex of each individual.

Parentage and Relatedness

Genotypes were identified for all individuals in the population using 14 polymorphic microsatellite markers previously isolated in the Seychelles warbler (Richardson et al. 2000). Parentage was determined (following Richardson et al. 2001) for all offspring sampled between 1997–1999 as part of another study (D. Richardson, J. Komdeur, and T. Burke, unpubl. ms.). An individual pair-wise coefficient of relatedness (r) based on microsatellite genotype similarity was calculated using the KINSHIP program (Goodnight and Queller 1999) for all within-territory dyads of (1) subordinate bird-nestling, (2) subordinate bird-primary female, and (3) subordinate bird-primary male. Using KINSHIP the mean pair-wise relatedness between all random individuals in the population was set to zero. The mean subordinate bird-nestling r was calculated when more than one descendant or more than one nondescendant nestling existed in the same nest. Each subordinate bird observed at sampled nests between 1997 and 1999 was used only once in the calculations of subordinate-offspring relatedness.

Territory Quality

As the warblers are insectivorous, taking 98% of their insect food from leaves (Komdeur 1991), the quality of a territory depends on the insect prey available, amount of foliage, and territory size. Territory quality (t) was determined in each breeding season following Komdeur (1994b) and can be expressed in terms of mean number of prey insects available by the following equation:

$$t = a \sum_{x=1}^{12} (c_x i_x) \quad (1)$$

where a is the mean territory size (hectares), c_x is the total foliage cover for plant species x and i_x is the mean monthly insect total for plant species x per unit leaf area (one square

decimetre). Territory quality was assessed monthly within each breeding season. Territories were divided into three territory quality categories: low (0–15), medium (15–30), or high quality (> 30) following Komdeur (1994b).

Fitness Benefits

Fitness benefits were measured using the number of offspring sampled for genetic analysis in the week prior to fledging. Failed nests were excluded from the analysis, because it was impossible to determine patterns of parentage and relatedness at nests where no offspring were blood-sampled. The effect of subordinates on the probability of nest failure was tested in a separate analysis (see results).

We calculated the indirect benefits (I) gained by each subordinate in terms of the equivalent number of descendent offspring (offspring equivalents) per breeding season using the formula: $I = 2r((O - O')/N)$, where O is the number of offspring (excluding direct subordinate parentage) fledged on a subordinate's territory, O' is the mean number of offspring fledged on territories without subordinates, N is the number of subordinates on a given territory, and r is the relatedness of the subordinate to the nondescendent offspring within the territory (multiplied by two to convert the figure into units equivalent to the number of offspring produced as first order relatives with $r = 0.5$). For the calculation of O', the mean number of offspring produced on territories without subordinates was calculated using all territories observed between 1997 and 1999 together because we found no effect of territory quality or breeding season on number of offspring produced. Direct breeding benefits were calculated as the sum of the coefficients of relatedness between the subordinate and its offspring, multiplied by two. The combined benefits were simply the sum of direct and indirect breeding benefits for each subordinate. Neither the direct nor indirect benefits were normally distributed.

Statistical Analysis

Transformations and nonparametric tests were used where appropriate. Tests were performed using SPSS 10.7 (SPSS 1999). All tests are two-tailed and corrected for continuity or tied ranks, as appropriate. Means are given ± SD. Five individuals (all females) were observed as subordinates in two breeding seasons; therefore, to avoid pseudoreplication, when calculating relatedness and fitness benefits we used the mean value across seasons for these individuals.

RESULTS

Relatedness

Sixty-three individuals were observed taking part in 68 successful helping events (a subordinate in a territory with at least one fledgling in a single breeding season), producing a total of 101 nestlings. Subordinates were significantly more likely to be female (43/63 = 68%) than male (20/63 = 32%; $\chi^2 = 8.41$, df = 1, $P < 0.01$). The number of territories with or without subordinates in each breeding season is shown in Table 1. Five individuals (all females) occurred as subordinates in two separate breeding seasons and both helping events are included in Table 1; however, each year was tested separately to avoid pseudoreplication.

TABLE 1. The number of territories in each breeding season between 1997 and 1999 that contained either no subordinates or at least one subordinate. The proportions of (1) all territories or (2) only territories that successfully produced nestlings are given.

	All territories			Successful territories		
Season	N[1]	Subs[2]	% Subs	n	Subs	% Subs
1997[a]	114	40	35	38	14	37
1997[b]	111	37	33	36	9	35
1998[a]	109	28	26	48	12	25
1998[b]	107	26	24	16	2	23
1999[a]	108	29	27	56	13	23
1999[b]	108	32	32	27	8	30
Mean ± SD			29 ± 0.01			25 ± 0.03

[1] Number of territories.
[2] Territories with subordinates.
[a] July–September breeding season.
[b] December–March breeding season.

Territories with subordinates were no more likely to produce offspring successfully than were territories without subordinates (Table 1, each year tested separately). Of the 68 successful helping events, 37 cases involved only one subordinate on a territory, while 14 sets of two subordinates and one set of three subordinates also occurred. Of the 63 individual birds, 20 subordinate females and three subordinate males gained parentage within their territory. Subordinate females were more likely to be parents than were males (20/43 vs. 3/20; Fisher's exact test, $P = 0.024$). Twelve subordinate females had both descendent and nondescendent kin in the same nest (which contained multiple offspring), whereas 8/20 subordinate females and all three subordinate males that gained parentage were the sole parent of their sex at a given nest.

As expected, there was no difference between the relatedness of subordinate males and females to descendent kin (0.44 ± 0.23 vs. 0.49 ± 0.10; $t = -0.40$, df = 21, $P = 0.70$). Overall, subordinate parents had a mean relatedness to descendent kin not significantly different than expected for a first order relative (observed vs. expected: 0.44 ± 0.22 vs. 0.5, independent sample test, $t = -1.24$, df = 22, $P = 0.23$). For females there was no significant difference between subordinate parents and subordinate nonparents in their relatedness to nondescendent offspring, primary females or primary males (Table 2). Because there were only three cases of subordinate males gaining paternity in the territory and none of these had nondescendent offspring in the same nest, we could not test for differences between direct and nondescendent kin. Male subordinates also did not appear to differ in their relatedness to either the primary female or the primary male.

Because there was no significant difference in how related subordinate parents and nonparents were to other adult members within the group, these two categories were combined together. Female subordinates were significantly related (greater than zero) to nondescendent offspring, to the primary female, and to the primary male within their territory (Table 2). Male subordinates were significantly related to the primary female but not to the nondescendent offspring nor to

TABLE 2. Mean (±SD) pair-wise relatedness (*r*) coefficients between subordinates and the primary female, primary male and nondescendent offspring within the territory. The *r*-values for subordinates with, and without, direct parentage in the nest were first compared, then all subordinates combined to determine if the *r* differed from that expected for unrelated dyads of individuals ($r = 0$).

Relatedness of subordinates to other group members	Subordinates							
	With parentage		Without parentage			All subordinates		Versus unrelated[4]
	r[1]	n[2]	r	n	t[3]	r	n	
Female vs.								
ND[5] offspring	0.08 ± 0.18	12[6]	0.16 ± 0.25	23	0.85 ns	0.13 ± 0.23	35	3.30**
Primary female	0.12 ± 0.28	20	0.23 ± 0.33	23	1.20 ns	0.17 ± 0.44	43	3.76***
Primary male	0.19 ± 0.19	20	0.14 ± 0.28	23	0.61 ns	0.16 ± 0.24	43	4.47***
Male vs.								
ND offspring	—	0	0.08 ± 0.25	17	—	0.08 ± 0.25	17	1.29 ns
Primary female	0.03 ± 0.30	3	0.13 ± 0.21	17	0.71 ns	0.12 ± 0.22	20	2.43*
Primary male	0.01 ± 0.28	3	0.05 ± 0.28	17	0.09 ns	0.05 ± 0.22	20	1.07 ns

[1] Mean pairwise relatedness.
[2] Number of individual subordinates.
[3] *t*-test of the difference in *r* between subordinates with and without direct parentage.
[4] *t*-test to determine if the *r* differed from zero.
[5] Nondescendent offspring.
[6] 8 female subordinate parents had no nondescendent kin in the territory.
* $P < 0.050$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 3. The mean (±SD) number of offspring produced by Seychelles warbler breeding pairs in territories without subordinates that successfully bred, for each breeding season between 1997 and 1999. The median number of offspring was 1 and the range was 1–2 in all cases. Statistical significance of the difference between territorial qualities in each year (and for all years combined) was assessed using the Kruskal Wallis χ^2 approximation. Territories were divided into three territory quality categories: low (1), medium (2), or high quality (3).

Breeding season	Territorial quality	n[1]	No. fledglings	χ^2
1997[a]	1	0	—	
	2	3	1.00 ± 0.00	
	3	7	1.14 ± 0.38	0.43 ns
	Total	10	1.10 ± 0.32	
1997[b]	1	3	1.00 ± 0.00	
	2	3	1.00 ± 0.00	
	3	10	1.00 ± 0.00	0.00 ns
	Total	16	1.00 ± 0.00	
1998[a]	1	0	—	
	2	9	1.00 ± 0.00	
	3	15	1.07 ± 0.26	0.60 ns
	Total	24	1.04 ± 0.20	
1998[b]	1	2	1.00 ± 0.00	
	2	3	1.00 ± 0.00	
	3	7	1.14 ± 0.38	0.71 ns
	Total	12	1.08 ± 0.29	
1999[a]	1	3	1.00 ± 0.00	
	2	8	1.13 ± 0.35	
	3	18	1.17 ± 0.38	0.60 ns
	Total	29	1.14 ± 0.35	
1999[b]	1	0	—	
	2	3	1.00 ± 0.00	
	3	8	1.13 ± 0.35	0.38 ns
	Total	11	1.09 ± 0.30	
Total	1	8	1.00 ± 0.00	
	2	29	1.03 ± 0.19	
	3	65	1.11 ± 0.31	2.20 ns
	Total	102	1.08 ± 0.27	

[1] Number of territories.
[a] July–September breeding season.
[b] December–March breeding season.

the primary male (Table 2). When the sexes were compared (see Table 2), female and male subordinates did not differ significantly in their relatedness to the primary female (*t*-test = 0.74, df = 61, $P < 0.46$) to the primary male (*t*-test = 1.76, df = 50, $P = 0.08$) or to non-descendent offspring (*t*-test = 0.69, df = 61, $P = 0.49$). Both female subordinates ($t = 0.240$, df = 42, $P = 0.81$), and male subordinates ($t = 1.22$, df = 19, $P = 0.24$), were equally related to the primary female and male in the territory (Table 2). Finally, both female ($t = 9.55$, df = 34, $P < 0.001$), and male ($t = 6.83$, df = 16, $P < 0.001$) subordinates were significantly less related to the nondescendent offspring in the territory than expected for first order relatives ($r = 0.50$).

The Benefits of Cooperative Breeding

The number of young produced on successful territories without subordinates did not differ significantly in relation to territory quality either within each breeding season, or when all breeding seasons were combined (Table 3). After combining all different quality territories, the median number of offspring fledged on successful territories without subordinates did not differ significantly between breeding seasons (Table 3; Kruskal Wallis $\chi^2 = 3.29$, df = 5, $P = 0.66$). Therefore, the data from all three years were combined and a mean fledgling success was calculated for pairs that bred in more than one season. From a total of 102 successful breeding attempts on 62 different territories, we calculate that a mean of 1.08 (± 0.27) fledglings were produced per territory without subordinates, per breeding season.

In 50 different cooperatively breeding groups (territories) containing 63 different subordinates, in total 101 offspring were produced, and 26 of these were produced by direct subordinate parentage. After excluding direct parentage, the mean number of extra offspring produced on a territory per subordinate (see Methods) was significantly greater than zero (0.18 ± 0.50, $n = 63$ subordinates; one sample *t*-test, $t = 2.76$, df = 62, $P < 0.01$). The relatedness of each subordinate

TABLE 4. The mean (±SD) fitness benefits of cooperative breeding gained by female and male subordinates in the Seychelles warbler (1997–1999). Statistical significance assessed by Mann-Whitney *Z* statistic.

Benefits	All birds $n = 63$	Females $n = 43$	Males $n = 20$	Between sex (Z)
Direct breeding	0.36 ± 0.58	0.46 ± 0.63	0.14 ± 0.35	2.29**
Indirect	0.06 ± 0.23	0.07 ± 0.26	0.04 ± 0.17	0.21
Combined	0.38 ± 0.57	0.48 ± 0.62	0.18 ± 0.38	1.80*
Direct vs. indirect (Z)	4.85***	4.22***	2.39**	

* $P = < 0.100$, ** $P < 0.050$, *** $P < 0.001$.

to the offspring within their territory was then used to calculate the indirect benefits per subordinate (Table 4).

Our results show that for subordinates the direct benefits of cooperative breeding were significantly greater than indirect benefits. The combined benefits of being a subordinate corresponded to 0.38 ± 0.57 of an offspring per breeding season at successful nests. Female subordinates were more likely than male subordinates to gain direct parentage within the nest, whereas male subordinates were, on average, less related to the nondescendent offspring in the nest than female subordinates (Table 2); therefore, the benefits of cooperative breeding were examined for each sex separately (Table 4). Direct benefits were significantly higher for females than males, but there was no significant difference for indirect benefits. The combined benefits also tended to be higher for females (not significant, $P = 0.072$). In both sexes direct benefits were significantly higher than indirect benefits, although this difference was more extreme in females, which gained significantly more direct benefits than males (Table 4, Fig. 1). There was no effect of the number of subordinates present in a territory on the direct (Kruskal Wallis $\chi^2 = 0.42$, df = 2, $P = 0.81$) or indirect benefits (Kruskal Wallis $\chi^2 = 4.17$, df = 2, $P = 0.12$) gained by a subordinate.

None of the 43 subordinate females gained reproductive success through egg dumping in other territories and none of the 20 male subordinates gained extrapair fertilization with females from other groups.

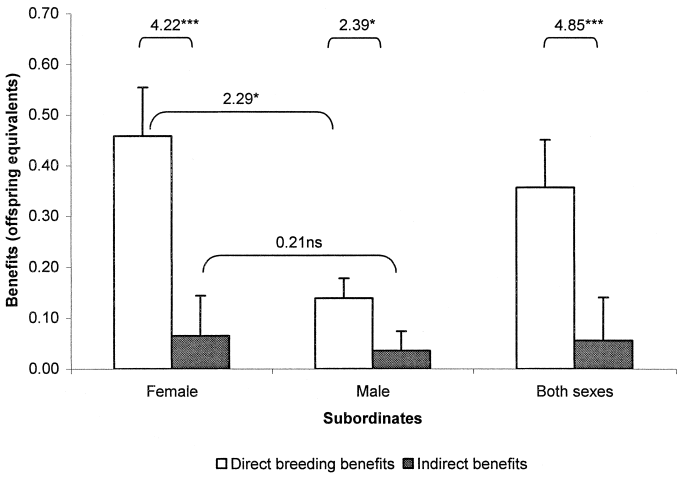

FIG. 1. The fitness benefits of cooperative breeding gained by female and male subordinates in the Seychelles warbler (1997–1999). Statistical significance assessed by Mann-Whitney *Z*-statistic. Both female ($n = 43$) and male ($n = 20$) subordinates gain significantly higher direct breeding benefits (open columns) compared to indirect benefits (filled columns). Direct breeding benefits are significantly higher in females than in males, but there is no significant difference between the sexes in indirect breeding benefits. Error bars represent one standard error. See Table 4 for details.

DISCUSSION

Kinship between Subordinates and Offspring

Previous work on fitness benefits in the Seychelles warbler (Komdeur 1994a) was based on an assumption that subordinates were the previous offspring of the primary individuals within a territory and, therefore, would be siblings of the nondescendants that they helped. In the present study, the direct offspring of subordinates were identified by parentage analysis and then the level of relatedness between subordinates and nestlings was calculated just for nondescendent offspring. In both sexes the mean subordinate-nondescendent offspring relatedness was significantly lower than expected for first order relatives. Female subordinates were, on average, significantly related to nondescendent offspring ($r = 0.13$), but at a level only approximating to cousins. Male subordinates were not, on average, significantly related to the nondescendent offspring produced within their territory ($r = 0.08$).

Although Seychelles warblers normally become subordinates on their natal territory, the level of relatedness between subordinates and nondescendent offspring is low as a result of two factors. First, extragroup paternity is frequent in the Seychelles warbler (40%; Richardson et al. 2001) and thus subordinates often have different fathers to the nondescendent offspring in the nest. Second, mixed parentage often occurs within a nest, with any combination of parents or stepparents and siblings, half-siblings, or less related individuals sharing in reproduction. The above result indicate that unless extrapair paternity, intraspecific brood parasitism, and direct reproduction by subordinates have been explicitly ruled out for a studied species, assessments of indirect benefits based on assumed levels of relatedness between subordinates and offspring could be inaccurate and misleading. Other studies have also shown that the actual levels of relatedness can be surprisingly different from those originally predicted, for example, in white-fronted bee-eaters, *Merops bullockoides* (Emlen and Wrege 1989; Emlen 1995), superb fairy wrens, *Malurus cyaneus* (Dunn et al. 1995), and splendid fairy wrens, *Malurus splendens* (Brooker et al. 1990; Rowley and Russell 1990). Clearly care must be taken when appraising the results of studies for which pair-wise relatedness has not been verified using molecular techniques.

Female and male subordinates do not differ significantly in how related they are to the primary female, to the primary

male, or to the nondescendent offspring within a territory. However, the relatedness of male subordinates to other group members is consistently lower than the relatedness of female subordinates to other group members; female subordinates are significantly related to nondescendent offspring and to the primary male and female in the territory, but male subordinates are significantly related only to the primary female. The lower mean relatedness of male compared to female subordinates is probably due to their weaker philopatric behavior—females are more likely than males to become subordinates (88% of helpers are female, Komdeur 1996a) and whereas females always become subordinates on their natal territory, males are occasionally observed as subordinates on nonnatal territories (5/20 males vs. 0/43 females; $\chi^2 = 8.51$, $P < 0.05$).

Other studies of cooperative breeding species have shown that subordinates gain parentage (e.g., Rabenold et al. 1990; Keane et al. 1996; Jamieson 1997; for joint nesting in female birds, see review by Vehrencamp 2000). As reproductive skew models predict (Emlen 1982, 1995; Vehrencamp 1983; Keller and Reeve 1994), levels of reproductive sharing within cooperative breeding systems do generally appear to be negatively correlated with the relatedness between group members of the same sex (Packer et al. 1991; Keane et al. 1996; Jamieson 1997; Whittingham et al. 1997; Lundy et al. 1998; Heinsohn et al. 2000; Vehrencamp 2000). Our study found that the relatedness between female subordinates and primary birds in a territory did not affect the subordinates' probability of gaining maternity. Female subordinates who did and did not gain parentage were equally related to the primary male and female in the territory. Unfortunately the small number of male subordinates within this study precluded any meaningful analysis of the link between parentage and group relatedness for males. Further work is needed to model the Seychelles warbler system in relation to reproductive skew theory.

The Benefits of Cooperative Breeding

Subordinate Seychelles warblers (especially females) frequently gained direct parentage within their own group (Richardson et al. 2001; this study). Reproductive skew theory (Emlen 1982, 1995; Vehrencamp 1983; Keller and Reeve 1994) suggests that a dominant will share reproduction with a subordinate if it realizes a higher inclusive fitness in the presence of the subordinate. In the present study we show that, on average, the presence of a subordinate upon a territory resulted in a significant increase in the number of offspring produced by the territory (0.18 extra offspring per subordinate), even after removing the direct offspring produced by subordinates. In addition, the dominant pair will also gain indirect benefits through the related subordinates' direct reproduction. Within the Seychelles warbler the primary (dominant) females within a group do, therefore, gain inclusive fitness benefits by allowing subordinate females to breed.

Many (47%) female subordinates gained direct parentage within the group, resulting in an intermediate level of female reproductive skew, which challenges the dichotomous ''helper-at-the-nest vs. joint nesting'' classification for avian cooperative breeders (Vehrencamp 2000). Subordinate male Seychelles warblers also gained direct benefits through parentage within the group (15% of male subordinates), although significantly less often than females. Subordinate paternity has been shown to occur (normally at low levels) in some species with male subordinates (e.g., stripe-backed wrens, *Campylorhynchus nuchalis* [Rabenold et al. 1990]; Arabian babblers, *Turdoides squamiceps* [Lundy et al. 1998]; bicolored wrens, *Campylorhynchus griseus* [Haydock et al. 1996]; and western bluebirds, *Sialia mexicana* [Dickinson and Akre 1998]).

Subordinate Seychelles warblers do not gain fitness benefits through direct reproductive success on territories other than their own. Egg dumping between groups does not occur in the Seychelles warbler and subordinate male warblers do not increase their reproductive success through extragroup paternity, because all extra-group offspring are sired by dominant breeding males (Richardson et al. 2001, this study).

Seychelles warblers also gain indirect fitness benefits through cooperative breeding. Previous work on the same population of Seychelles warblers showed an increased production of offspring by the recipients of help (Komdeur 1994b). However, this earlier study did not control for direct subordinate parentage. Our present study confirms that subordinates enhanced the production of nondescendent offspring in a territory (0.18 per subordinate), but because of low levels of relatedness between subordinates and nondescendent kin (especially for males), this translated into small indirect fitness benefit gains for subordinates (mean offspring equivalents: females = 0.07 ± 0.26, males = 0.04 ± 0.17). Furthermore, as male subordinates are not more related to the offspring in the territory than expected by chance, indirect benefits could be considered to be nonexistent for males.

Our study also found that the relationship between subordinates and nondescendent offspring could be anywhere between unrelated to that of full siblings. Komdeur (1994a) previously showed that subordinate Seychelles warbler use associative learning rules to direct helping behavior, adjusting their provisioning levels to the continued presence of their own putative parents within the territory. Further work is required to determine whether or not this strategy allows subordinate Seychelles warblers to accurately maximize indirect benefits by preferentially helping more closely related kin.

The present study shows that in the Seychelles warbler the direct breeding benefits of cooperative breeding are, on average, 6.0 times greater than the indirect benefits (offspring equivalents; 0.36 vs. 0.07, respectively). However, one criticism of our study is that by only measuring the production of nestlings we did not take into account future indirect fitness benefits (Mumme et al. 1989, reviewed in Emlen 1995; Cockburn 1998). Postfledgling survival is greater in territories with subordinates in the Seychelles warbler (Komdeur 1994a), but this will equally benefit subordinates' descendent and nondescendent offspring and would not change the relative importance of direct and indirect benefits.

Future indirect benefits occur when subordinates increase the survival and future reproductive success of closely related primary birds and have been shown to account for between 29–49% of the total gain in indirect fitness in certain cooperative breeding systems (Mumme et al. 1989). In the Sey-

chelles warbler, subordinates (especially males) have a relatively low average coefficient of relatedness to the primary birds (see Table 2), and there is no evidence that subordinates increase the survival or future reproductive success of primary birds (Komdeur 1994b). Future indirect fitness benefits are, therefore, unlikely to be high, but even a 50% gain in indirect benefits would make little difference to the overriding dominance of direct benefits within the Seychelles warbler.

Future direct benefits are, on the other hand, important in the Seychelles warbler. Komdeur (1996b) showed that the acquisition of parenting skills led to female subordinates being superior breeders when they acquire a territory (although the present study suggests they may become superior breeders as a result of direct breeding experience rather than through helping). Subordinates may also gain by budding off part of a territory that has been enlarged through group augmentation (Komdeur and Edelaar 2001). The inclusion of the extra direct benefits that subordinates gain would only provide further support for the predominant role of direct benefits in the evolution and maintenance of cooperative breeding in the Seychelles warbler.

Subordinates Sex Bias

The Seychelles warbler is atypical in cooperative breeding birds in that it has male dispersal and female-biased helping (Komdeur 1992, 1996a). Various explanations exist for sex bias dispersal, and studies have shown that the sexes may obtain different benefits from helping (reviewed in Cockburn 1998) or that the cost of helping may be significant and differ between the sexes (Rabenold et al. 1990; Heinsohn and Cockburn 1994; reviewed in Heinsohn and Legge 1999). Our results show that, as predicted, female subordinates remaining on the natal territory gain significantly more benefits than male subordinates in the Seychelles warbler and this may explain why most subordinates are female.

The differing costs of dispersal for each sex could also result in sex-biased dispersal patterns, however there is no evidence from the long-term study on the Seychelles warbler that male and females face different dispersal costs (J. Komdeur, pers. comm.)

It may be that primary birds preferentially accept female subordinates. Indeed previous studies have shown that primary females skew the sex ratio of eggs so as to produce a greater proportion of female offspring, which will become subordinates in subsequent years (Komdeur et al. 1997). This preference for female subordinates can be explained by the greater benefits accruing to primary birds through female rather than male subordinates. With female subordinates the primary birds gain both an increase in their own productivity and also indirect benefits associated with subordinate females breeding. Male subordinates do not increase the group's productivity by laying eggs in the nest as female subordinates do, but rather compete with the dominant male for the fertilization of these eggs.

Other studies show that help is often not beneficial when males help, but is when females help (Cockburn 1998). For example, in splendid fairy wrens, *Malurus splendens*, female subordinates result in an increase in productivity but male subordinates do not (Brooker and Rowley 1995). In theory it is possible that in the Seychelles warbler, the primary birds (especially males who may lose within-group paternity) drive male subordinates out of the territory. No aggressive behavior has been observed between primary birds and male or female subordinates (J. Komdeur, pers. obs.), but further work is required to investigate the hypothesis.

Sex-biased dispersal could also be a mechanism to avoid inbreeding avoidance, however other factors would still determine which sex dispersed. In the Seychelles warbler dispersal as an inbreeding avoidance mechanism is neither thorough, nor completely successful as both sexes do remain as subordinates and inbreeding is not completely avoided (D. Richardson, J. Komdeur, and T. Burke, unpubl. ms.).

Conclusion

Our results change our perception of the relative importance of direct and indirect benefits in the evolution and maintenance of cooperative breeding in the Seychelles warbler. We show that subordinates (mainly females) remaining on their natal territories frequently gained direct parentage. Subordinate breeding, combined with high levels of extragroup paternity, resulted in lower than predicted levels of relatedness between subordinates and nondescendent offspring. Direct benefits, which were 6.0 times higher than indirect kin benefits, are the main factor selecting for cooperative breeding in the Seychelles warbler. Direct benefits were higher in both female and male subordinates but, as predicted, female subordinates gained significantly higher direct fitness benefits than males. This may explain why primary females often produce female eggs, in order to have female subordinates in the Seychelles warbler.

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Literature Cited

Arnold, E. K., and I. P. F. Owens. 1998. Cooperative breeding in birds: a comparative test of the life history hypothesis. Proc. R. Soc. Lond. B. 265:739–745.

Brooker, M., and I. Rowley. 1995. The significance of territory size and quality in the mating strategy of the splendid fairy-wren. J. Anim. Ecol. 64:614–627.

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

Brown, J. L. 1987. Helping and communal breeding in birds: ecology and evolution. Princeton Univ. Press, Princeton, NJ.

Burke, T. A. 1989. DNA Fingerprinting and other methods for the studying of mating success. Trends Ecol. Evol. 4:139–144.

Cant, M. A., and J. Field. 2001. Helping effort and future fitness

in cooperative animal societies. Proc. R. Soc. Lond. B. 268: 1959–1964.
Clutton-Brock, T. H., P. N. M. Brotherton, M. J. O'Riain, A. S. Griffin, D. Gaynor, L. Sharpe, R. Kansky, M. B. Manser, and G. M. McIlrath. 2000. Individual contributions to babysitting in a cooperative mongoose, *Suricata suricatta*. Proc. R. Soc. Lond. B. 267:301–305.
Clutton-Brock, T. H., P. N. M. Brotherton, M. J. O'Riain, A. S. Griffin, D. Gaynor, R. Kansky, L. Sharpe, and G. M. McIlrath. 2001. Contributions to cooperative rearing in meerkats. Anim. Behav. 61:705–710.
Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. Annu. Rev. Ecol. Syst. 29:141–177.
Dunn, P. O., A. Cockburn, and R. A. Mulder. 1995. Fairy-wren helpers often care for young to which they are unrelated. Proc. R. Soc. Lond. B. 259:339–343.
Dickinson, J. L., and J. J. Akre. 1998. Extra-pair paternity, inclusive fitness, and within-group benefits of helping in western bluebirds. Mol. Ecol. 7:95–105.
Du Plessis, M. A. 1993. Helping behavior in cooperatively breeding green woodhoopoes—selected or unselected trait. Behaviour 127:49–65.
Emlen, S. T. 1982. The evolution of helping. 1. An ecological constraints model. Am. Natur. 119:29–39.
———. 1995. An evolutionary theory of the family. Proc. Natl. Acad. Sci. USA. 92:8092–8099.
———. 1997. Predicting family dynamics in social vertebrates. Pp. 228–253 in J. R. Krebs and N. B. Davies, eds. Behavioural ecology: an evolutionary approach. Blackwell Science, Oxford, U.K.
Emlen, S. T., and P. H. Wrege. 1989. A test of alternate hypotheses for helping-behavior in white-fronted bee-eaters of Kenya. Behav. Ecol. Sociobiol 25:303–319.
Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Mol. Ecol. 8:1231–1234.
Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Mol. Ecol. 7:1071–1075.
Hamilton, W. D. 1964. The genetical evolution of social behaviour. I and II. J. Theor. Biol. 7:1–52.
Hatchwell, B. J., and J. Komdeur. 2000. Ecological constraints, life history traits and the evolution of cooperative breeding. Anim. Behav. 59:1079–1086.
Haydock, J., P. G. Parker, and K. N. Rabenold. 1996. Extra-pair paternity in the cooperatively breeding bicolored wren. Behav. Ecol. Sociobiol 38:1–16.
Heinsohn, R., and A. Cockburn. 1994. Helping is costly to young birds in cooperatively breeding white-winged choughs. Proc. R. Soc. Lond. B. 256:293–298.
Heinsohn, R., and S. Legge. 1999. The cost of helping. Trends Ecol. Evol. 14:53–57.
Heinsohn, R., P. Dunn, S. Legge, and M. C. Double. 2000. Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs. Proc. R. Soc. Lond. B. 267:243–249.
Jamieson, I. 1991. The unselected hypothesis for the evolution of helping-behavior—too much or too little emphasis on natural selection. Am. Nat. 138:271–282.
———. 1997. Testing reproductive skew models in a communally breeding bird, the pukeko, *Porphyrio porphyrio*. Proc. R. Soc. Lond. B. 264:335–340.
Jennions, M. D., and D. W. Macdonald. 1994. Cooperative breeding in mammals. Trends Ecol. Evol. 9:89–93.
Keane, B., S. R. Creel, and P. M. Waser. 1996. No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. Behav. Ecol. 7:480–489.
Koenig, W. D., F. A. Pitelka, W. J. Carmen, R. L. Mumme, and M. T. Stanback. 1992. The evolution of delayed dispersal in cooperative breeders. Q. Rev. Biol. 67:111–150.
Keller, L., and H. K. Reeve. 1994. Partitioning of reproduction in animal societies. Trends Ecol. Evol. 9:98–102.
Komdeur, J. 1991. Cooperative breeding in the Seychelles warbler. Ph.D. diss. Cambridge University, Cambridge, U.K.
———. 1992. Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. Nature 358:493–495.
———. 1994a. The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). Proc. R. Soc. Lond. B. 256:47–52.
———. 1994b. Experimental evidence for helping and hindering by previous offspring in the cooperatively breeding Seychelles warbler *Acrocephalus sechellensis*. Behav. Ecol. Sociobiol 34: 175–186.
———. 1996a. Facultative sex ratio bias in the offspring of Seychelles warblers. Proc. R. Soc. Lond. B. Biol. Sci. 263:661–666.
———. 1996b. Influence of helping and breeding experience on reproductive performance in the Seychelles warbler: a translocation experiment. Behav. Ecol. 7:326–333.
Komdeur, J., and P. Edelaar. 2001. Male Seychelles warblers use territory budding to maximize lifetime fitness in a saturated environment. Behav. Ecol. 12:706–715.
Komdeur, J., S. Daan, J. Tinbergen, and C. Mateman. 1997. Extreme adaptive modification in the sex ratio of Seychelles warbler's eggs. Nature 385:522–525.
Lundy, K. J., P. G. Parker, and A. Zahavi. 1998. Reproduction by subordinates in cooperatively breeding Arabian babblers is uncommon but predictable. Behav. Ecol. Sociobiol 43:173–180.
McRae, S. B., and W. Amos. 1999. Can incest within cooperative breeding groups be detected using DNA fingerprinting? Behav. Ecol. Sociobiol 47:104–107.
Magrath, R. D., and L. A. Whittingham. 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.
Mumme, R. L. 1992. Delayed dispersal and cooperative breeding in the Seychelles warbler. Trends Ecol. Evol. 7:329–331.
Mumme, R. L., W. D. Koenig, and F. L. W. Ratnieks. 1989. Helping behavior, reproductive value, and the future component of indirect fitness. Anim. Behav. 38:331–343.
Packer, C., D. A. Gilbert, A. E. Pusey, and S. J. Obrien. 1991. A molecular genetic-analysis of kinship and cooperation in African lions. Nature 351:562–565.
Parker, P. G., T. C. Jones, J. Haydock, J. L. Dickinson, and B. D. Worden. 1999. Multilocus minisatellite DNA fingerprinting and cooperative breeding. Behav. Ecol. Sociobiol 47:108–111.
Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1993. Microsatellites and kinship. Trends Ecol. Evol. 8:285–288.
Rabenold, K. N. 1985. Cooperation in breeding by nonreproductive wrens—kinship, reciprocity, and demography. Behav. Ecol. Sociobiol 17:1–17.
Rabenold, P. P., K. N. Rabenold, W. H. Piper, J. Haydock, and S. W. Zack. 1990. Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. Nature 348:538–540.
Reyer, H. U. 1980. Flexible helper structure as an ecological adaptation in the pied kingfisher (*Ceryle rudis rudis* L). Behav. Ecol. Sociobiol. 6:219–227.
Richardson, D. S., F. L. Jury, D. A. Dawson, P. Salgueiro, J. Komdeur, and T. Burke. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. Mol. Ecol. 9:2226–2231.
Richardson, D., F. Jury, K. Blaakmeer, J. Komdeur, and T. Burke. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). Mol. Ecol. 10:2263–2273.
Rowley, I., and E. Russell. 1990. Philandering: a mixed mating strategy in the splendid fairy-wren, *Malurus splendens*. Behav. Ecol. and Sociobiol. 27:431–437.
Russell, A. F., and B. J. Hatchwell. 2001. Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. Proc. R. Soc. Lond. B. 268:2169–2174.
Sherley, G. H. 1990. Cooperative breeding in riflemen (*Acanthissitta chloris*) benefits to parents, offspring and helpers. Behaviour 112:1–22.
Stacey, P. B., and W. D. Koenig. 1990. Cooperative breeding in birds: long-term studies of ecology and behavior. Cambridge Univ. Press, Cambridge, U.K.

SPSS. 10.7. 1999. Vers. 10.7. SPSS Inc, Chicago, IL.
Vehrencamp, S. L. 1983. A model for the evolution of despotic versus egalitarian societies. Anim. Behav. 31:667–682.
Vehrencamp, S. L. 2000. Evolutionary routes to joint-female nesting in birds. Behav. Ecol. 11:334–344.
West, S. A., and B. C. Sheldon. 2002. Constraints in the evolution of sex ratio adjustment. Science 295:1685–1688.
Whittingham, L. A., P. O. Dunn, and R. D. Magrath. 1997. Relatedness, polyandry and extra-group paternity in the cooperatively-breeding white-browed scrubwren (*Sericornis frontalis*). Behav. Ecol. Sociobiol. 40:261–270.
Zahavi, A. 1990. Arabian babblers: the quest for status in a cooperative breeder. Pp. 105–130 *in* P. B. Stacey and W. D. Koenig, eds. Cooperative breeding in birds. Cambridge Univ. Press, Cambridge, U.K.

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