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GRANDPARENT HELPERS: THE ADAPTIVE SIGNIFICANCE OF OLDER, POSTDOMINANT HELPERS IN THE SEYCHELLES WARBLER

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The possibility that older, often nonreproductive, individuals may engage in kin-directed cooperative behavior has been largely overlooked in the study of cooperative breeding. Here, we describe and investigate the adaptive significance of such "grandparent" helpers in the Seychelles warbler, the first bird species in which this phenomenon has been observed. On Cousin Island, over a period of 24 years, a significant proportion (13.7%) of females, but few males (3.0%), was deposed from dominant positions. Deposed females were replaced by related females. However there was no evidence that older, senescent females were stepping aside to gain greater fitness benefits by increasing the reproductive success of their offspring, rather than breeding themselves; deposed females were not postreproductive, nor was being deposed linked to age or reproductive senescence. Of the deposed females, 68% became subordinates and helped to raise group offspring, accounting for ca. 10% of subordinates in any year. Demoted females were related ($r = 0.24$) to the group offspring and, consequently, could gain indirect benefits through helping. As direct benefits appeared to be limited, we suggest that indirect benefits have driven the evolution of such "grandparent helpers." This study now provides evidence for a new route to cooperative breeding in birds.

KEY WORDS: **Cooperative breeding, direct benefits, grandparent helpers, indirect benefits, kin selection, relatedness, microsatellite markers.**

Cooperative breeding, where adult individuals (helpers) other than the dominant pair assist in the rearing of offspring from a single breeding attempt, has been well documented in birds and mammals (Brown 1987; Jennions and Macdonald 1994; Cockburn 1998; Koenig and Dickinson 2004) and in some fish (Taborsky 1994). In the vast majority of cooperatively breeding species, helpers are offspring that have delayed dispersal and remain within their natal group (reviewed in Jennions and Macdonald 1994; Emlen 1995; Koenig and Dickinson 2004). Consequently, helpers are normally assisting their parents and often accrue indirect fitness benefits by doing so (Emlen 1997; Dickinson and Hatchwell 2004).

In long-lived animals it has been suggested that the increase in mortality costs of reproduction with age may mean that older females are able to gain greater fitness benefits by increasing the survival and reproductive success of their offspring, rather than by breeding themselves (Williams 1957; Hamilton 1966). Indeed, it has been suggested that the abrupt cessation of reproduction documented in individuals of an advanced age in certain species, including humans, has evolved as part of this strategy (Williams 1957; Hawkes et al. 1998; Lee 2003). Older, often postreproductive, individuals have been documented to engage in kin-directed cooperative behavior in a very small number of mammalian species (Packer et al. 1998; Pavelka et al. 2002), and this behavior has, in cases, been shown to increase the reproductive success of the individual's offspring (Pavelka et al. 2002; Lahdenpera et al. 2004). However, the existence of what could be termed "grandparent" helpers appears to have been largely overlooked in the field of cooperative breeding outside humans. This may be because such helping occurs relatively infrequently or because, to our knowledge, no cases of demoted grandparent helpers have been identified in birds, the taxonomic group in which most studies on cooperative breeding systems have been undertaken (Koenig and Dickinson 2004). As cooperatively breeding bird species are often relatively long-lived (Arnold and Owens 1998; Hatchwell and Komdeur 2000), the potential for such behavior exists, and it may merely be that the majority of studies on such species have not progressed long enough for grandparent helpers to be identified. The concept of grandparent helpers is, however, of evolutionary importance, as it provides another route to cooperative breeding. Moreover, as the costs and benefits of being (or having) grandparent helpers may differ from those experienced for the more usual, prereproductive helpers, the investigation of such systems may shed new light on the factors driving the evolution of cooperative breeding.

The Seychelles warbler (*Acrocephalus sechellensis*) is a passerine bird endemic to the Seychelles Islands. The basic breeding unit is the pair, which defends a territory and normally remains together as long as both birds survive (Komdeur 2003). Annual survival is high, consequently birds may be paired up for as long as nine years (Komdeur 1994a), but there are negative effects of senescence on reproduction; for pair females, annual reproductive success increases quickly in the first three years, remains constant between four and six years of age, and decreases significantly thereafter (Komdeur 1996b). Subordinates (adult birds other than the dominant pair within a territory) are normally offspring that, because of a lack of suitable independent breeding opportunities, delay dispersal (Komdeur 1992). Delayed dispersal appears to be driven primarily by direct cobreeding benefits (Richardson et al. 2002) but female subordinates are also able to maximize the indirect fitness benefits they gain by using effective discrimination (Richardson et al. 2003a; Komdeur et al. 2004b). The higher overall level of benefits accrued by female, compared to male, subordinates is thought to explain why the majority of subordinates in the Seychelles warbler are female (Komdeur 1996a; Richardson et al. 2002).

Here, we report what is to our knowledge the first documented case of subordinate grandparent helpers in a bird species. In the Seychelles warbler, observations gathered over a number of years have suggested that dominant females may occasionally be deposed from their position, and whereas some of these deposed female birds become floaters, many remain in the territory as subordinates (D. S. Richardson, pers. obs.). Now by using the long-term dataset accumulated since 1981 on the Cousin Island population, in conjunction with molecular parentage and relatedness analysis, we aim to investigate the extent, and significance, of this surprising and novel behavior. First, we assess how often dominant individuals are deposed and whether this is equal for both males and females. We predict that males will be more resistant to being deposed than females, as they are less able to gain benefits through being subordinates than older females. Direct benefits are small, as subordinate males almost never gain parentage (Richardson et al. 2002). Furthermore, indirect benefits will also be small, as the high level of extra-pair paternity observed in this species (40%, Richardson et al. 2001) means that any offspring produced on the territory that take over as dominants will on average be less related to a deposed male than a deposed female. Second, we identify which females are deposed and what determines whether they become subordinates or floaters thereafter. Third, we examine what benefits a deposed female may gain by remaining on the territory as a subordinate. Finally, we assess whether this phenomenon may be an adaptive strategy in which, by stepping aside and becoming subordinate helpers for their offspring, older, senescent females may gain greater fitness benefits than by breeding themselves. If so, we predict that: (1) A female will only relinquish her dominant position to a closely related female. Therefore pairwise relatedness will be high between deposed females and the females that take over the dominant position in the territory, but lower between females that die while dominant and their subsequent female replacements. (2) A deposed females will be more likely to remain on the territory as a subordinate when replaced by a highly related female. Therefore relatedness between deposed females that become floaters and their replacements will be lower than between demoted subordinates and their replacements. (3) The probability and timing of a female being deposed, will be linked to reproductive senescence. (4) Demoted subordinates will actively assist the new dominant pair in raising offspring. (5) Demoted subordinates will be significantly related to the offspring they help raise.

Materials and Methods

STUDY POPULATION AND DATA COLLECTION

The Cousin Island (29 ha) population of Seychelles warblers has been monitored since 1981, and intensively so between 1985 and 2005. During this time, many of the birds (>96% of birds since 1997; Richardson et al. 2004) were individually color-ringed using a unique combination of three color rings and a British Trust for Ornithology metal ring. There is extremely limited migration on or off the island (Komdeur et al. 2004a) and all breeding attempts were monitored; therefore the complete life history, status, and putative pedigree of nearly all birds were known. During the breeding seasons (June–September, December–February) all territories (circa 115) were checked for the presence of ringed birds and nesting activity by following the resident female(s) for 30 min at least once per week. Thereafter, active nests were observed for a minimum of 10 min every 3–4 days through the breeding cycle. During these periods the first female to be seen and identified in the territory was followed and her behavior and the behavior and identity of individuals interacting with her were recorded. These detailed behavioral observations taken repeatedly over the breeding cycle were used to determine the status of all birds within each territory. The "dominant" male and female were defined as the primary, pair-bonded male and female in a territory, whereas the term "subordinate" included all other birds (>8 months old) resident in the territory. Dominance was based on the behavior of the male and female and not by parentage in the nest, which could be gained by both dominant and subordinate females (Richardson et al. 2001). In the Seychelles warbler, intense mate guarding occurs during the 10 days prior to egg laying (Komdeur et al. 1999). Dominant males follow dominant females around the territory, remaining in constant close proximity (within 5 m). Subordinate males do not mate guard, and dominant males do not intensively mate guard subordinate females (D. S. Richardson, pers. obs). Mate-guarding behavior therefore provides an accurate and rapid method to assess dominance, however a suite of other behaviors recorded across the breeding season (e.g., females instigating and dominating nest building) were also used to confirm dominance. Floaters were birds observed anywhere on the island that were not resident (consistently observed within a territorial group over a number of visits over a number of weeks) in any specific territory.

In the Seychelles warbler, additional birds in the territory do not always assist in the guarding and provisioning of offspring, hence the use of the term "subordinate" rather than "helper" for all additional birds. As part of the long-term monitoring of the Cousin population, all activity at focal nests was routinely observed for a minimum of 10 min every 3–4 days during nest building and incubation (14–16 days), and after hatching until fledging (18–20 days), to determine the presence and status of individuals within a territory (Richardson et al. 2002). Furthermore, at some focal nests included within a study on provisioning levels, focal watches of 90 min in duration were undertaken at least once during the incubation and provisioning periods to determine whether subordinates assisted in the rearing of offspring (Richardson et al. 2003a,b). During these periods, whether an individual (subordinate or dominant) either incubated eggs or provisioned chicks in the focal nest was recorded. These watches provided us with qualitative data on whether individual demoted subordinates helped towards a territorial breeding attempt.

Since 1993, blood samples (ca. 15 μl) have been 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 PCR method devised by Griffiths et al. (1998) was used to confirm the sex of each individual that had been blood sampled. As the warblers are insectivorous, taking 98% of their insect food from leaves (Komdeur 1991) the quality of a territory (tq) was measured as an index of insect prey availability following (Komdeur 1994b).

The long-term database on the Seychelles warbler population was used to identify all the ringed individuals on Cousin Island between 1981 and 2004 that held, and then lost, a dominant position on a territory. Only cases in which the group composition, individual status, and identity of birds within the territory had been clearly and reliably determined, through multiple observations over the course of the breeding season, were used in the analyses. Individuals that still held a dominant position at the end of this period were excluded, as they had not yet reached the end of their tenure. The postdominant status of each of these individuals was defined as one of three categories: (1) dead: individual that was never seen again after disappearing from its territory, (2) demoted subordinate: individual that lost its dominant position within a territory but remained on the same, or moved to another territory, where a dominant male and female pair were present, or (3) floater: individual that was observed in the years after losing its dominant position but that was not resident in any specific territory. Both males and females were included within this initial analysis so that rates of being deposed could be compared.

Tenure was defined as the number of years in which the individual was a dominant pair-bonded individual within a territory. The quality of the territory in the last year of the tenure was recorded. If this was not available then the territory quality in the preceding year was used. This could be justified, as annual variation in territory quality was relatively small (Komdeur 1996c). The individual was excluded from the analysis if a measure of quality was not available for the territory in either of these years. The age of each individual was determined. Exact ages were known for all individuals ringed as chicks, juveniles, or subadults (identified as being within first year of birth based on eye color), whereas minimal ages were determined for the small proportion of individuals ringed as adults. The number of territories per year that contained demoted female subordinates and the proportion of these demoted subordinates in relation to the overall number of subordinates was calculated in the years 1997, 1998, and 1999—three years for which we had exceptionally accurate data on the reproductive status of all individuals in all territories on Cousin (Richardson et al. 2002).

PARENTAGE AND RELATEDNESS

DNA extraction, genotyping, and parentage analysis was completed using the methodology described in Richardson et al. (2001) and CERVUS (Marshall et al. 1998). Parentage was analyzed for all offspring sampled between 1997 and 1999 ($n = 199$) in the course of other studies (Richardson et al. 2001, 2005). The software program KINSHIP (Goodnight and Queller 1999) was used to calculate the pairwise relatedness (r) between the deposed dominant female and the new dominant female, based on genotypic similarity at 14 microsatellite loci isolated in the Seychelles warbler (Richardson et al. 2000). Pairwise relatedness between the previous and the new dominant female could be calculated for 14 of the 19 demoted subordinates, seven of the nine cases in which the old females became floaters and for 48 of the 176 cases in which the female died before being replaced. The cases not included involved individuals that were either unringed, or for which we did not have a DNA sample from one or both of the females involved. For females that remained on a territory as a subordinate, the mean pairwise relatedness between each of them and all the offspring reared in the territory during their tenure as a demoted subordinate was also calculated. These relatedness values were tested against a background (calibrated) average population relatedness of zero, as is standard in KINSHIP.

STATISTICAL ANALYSES

Binary logistic regression analysis was used to test the effects of multiple independent terms on: (1) the probability of a female being deposed from her dominant position and (2) the probability of a deposed female remaining as a demoted subordinate or becoming a floater. The minimum adequate model was calculated following parsimonious streamlining (the stepwise backward removal of nonsignificant terms) of the original model. The cooperative breeding status of a territory, the presence/absence of male or female subordinates, the presence of a new dominant male, and whether a female had previous experience of being a subordinate before gaining a dominant position were entered as categorical variables, whereas all other terms (i.e., territory quality, age, relatedness) were entered as variates. Other measures of the cooperative-breeding status of the territory, including a binary cooperative versus noncooperative term, and the total number of subordinates in a territory, were used to replace the presence/absence of male or female subordinate terms to fully investigate the effect of such parameters. The Wald test statistic is reported for the predicted effect of each term on the model. Cox regression analysis was used to investigate the relationship between postdominant status and tenure, survival and life span.

Transformations and nonparametric tests were used where appropriate. Tests were performed using SPSS 10.7 (SPSS Inc 1999, Chicago, IL). All tests are two-tailed and corrected for continuity or tied ranks, as appropriate. Unless stated otherwise, means are given ± SD. Statistical significance was set at $P < 0.05$.

Results

From the data gathered between 1981 and 2004, we were able to unambiguously identify 204 cases in which females, and 263 cases in which males, held and then lost a dominant position. Significantly more females (28/204 = 13.7%) were deposed from their dominant position while still alive than males (8/263 = 3.0%; $\chi^2 = 18.43$, df = 1, $P < 0.001$). The other 176 females (86.3%) and 255 (97.0%) males were never observed after disappearing from their territory and were presumed to have died and then been replaced. Out of the 28 deposed females, 19 became subordinates on a territory after losing their dominant status (being demoted) whereas nine were recorded as floaters. Of the eight deposed males, only three were observed to become subordinates, whereas five were observed as floaters. There was no significant difference between the proportions of demoted males and females that became subordinates (3/8 = 37.5% vs. 19/28 = 67.9% respectively; Fisher exact test, $P = 0.22$) but the sample was very small for males. Hereafter all analyses were restricted to the females.

The analysis of the numbers of territories and female subordinates on Cousin during 1997, 1998, and 1999 shows that, on average, approximately 10% of all subordinates in any year were demoted females (Table 1).

LOSS OF DOMINANCE BY FEMALES

We used logistic regression analyses to investigate the factors that determined whether a female was deposed from her dominant position (deposed), as opposed to remaining dominant until her death (dead). The only factor that remained in the final model was whether the female had been a subordinate previously (prior to becoming a dominant bird), whereas female age, territorial quality, and the presence of male, or female, subordinates in the territory,

Table 1. The numbers (*N*) of territories and female subordinates on Cousin during 1997, 1998, and 1999, including the numbers and proportions of these that were demoted subordinates.

Year	*N* territories	*N* territories with female subordinates (% of territories)	Total *N* of female subordinates	*N* territories with demoted female subordinates (% of territories)	Total *N* of demoted female subordinates (% of subs)
1997	114	40 (35)	49	4 (3.5)	4 (8.2)
1998	109	28 (26)	37	4 (3.7)	4 (10.8)
1999	108	29 (27)	41	3 (2.8)	3 (9.7)
Mean	110	32 (29)	42	3.7 (3.3)	3.7 (9.6)

Table 2. **Logistic regression analyses investigating which factors influence: (a) whether a female was deposed from her dominant position (without including relatedness *r*); (b) whether a female was deposed from her dominant position, including only the significant terms from (a) and relatedness (*r*) between the old and the new dominant female; (c) whether deposed females remained as demoted subordinates or left and became floaters. The Wald test statistic and level of significance are shown for all terms included in the original model. Terms remaining in the minimum adequate model are highlighted in bold.**

Explanatory terms	Beta	SE	df	Wald	*P*
(a) Deposed versus dead females					
Previously subordinate	**2.37**	**0.60**	**1**	**15.91**	**$<$0.001**
Constant	**−0.30**	**0.30**	**1**		
Male subordinate present			1	2.22	0.137
Female subordinate present			1	1.14	0.259
Female age			1	0.96	0.328
Territory quality			1	0.11	0.744
(b) Deposed versus dead females including relatedness (r)					
Previously subordinate	**2.00**	**0.65**	**1**	**9.35**	**$<$0.001**
New-old female relatedness (*r*)	**2.79**	**1.42**	**1**	**3.85**	**$<$0.05**
Constant	**−4.58**	**1.20**	**1**		
(c) Demoted subordinate versus floater					
Territory quality	**−2.46**	**1.25**	**1**	**3.87**	**$<$0.05**
Constant	**1.10**	**0.95**	**1**		
Female age			1	1.71	0.191
Female subordinate present			1	0.77	0.379
Male subordinate present			1	1.07	0.302
New dominant male			1	0.25	0.874
Previously subordinate			1	0.01	0.931

Final models: (a) df = 2, 106, $r^2 = 0.18$; (b) df = 3, 65, $r^2 = 0.22$; (c) df = 2, 21, $r^2 = 0.45$

had no significant effect (Table 2a). The alternative factors, including the binary cooperative versus noncooperative term and the total number of subordinates in a territory, also had no effect when entered into the model. None of the other factors had a significant effect on the model, even when the previous subordinate experience term was dropped from the initial model ($P > 0.1$). No significant interactions between the terms in the models were detected. A post hoc univariate test confirmed that females that had previously been subordinates were significantly more likely to be deposed from their dominant position than were females that had never been subordinates (42% (22/52) vs. 8% (6/74); $\chi^2 = 22.85$, df = 1, $P < 0.001$).

Pairwise relatedness (r) between the previous and the new dominant female could only be calculated for 21 of the 28 cases where the dominant female was deposed and for 48 of the 154 cases in which the female died before being replaced. The initial model (Table 2a) was run without taking r into account, as its inclusion would dramatically reduce the degrees of freedom in the analysis. Relatedness was shown to have a significant effect and improve the final model when included with "previous subordinate" in a model with a reduced sample size (Table 2b). Deposed females were replaced by significantly more closely related females than were those females that died while dominant (Fig. 1).

It is possible that a female may lose her dominant position if the dominant male within the territory changes, that is, she loses her pair-bonded male and the new male pairs up with a different female. However, this does not appear to be the case; in

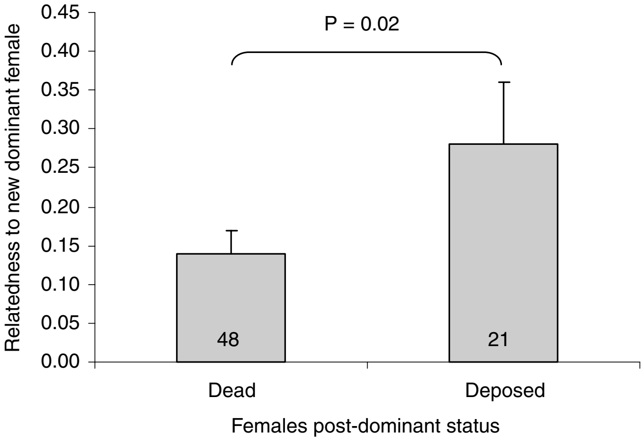

Figure 1. **Mean pairwise relatedness (*r*) between the two classes of replaced dominant female (dead vs. deposed) and the new dominant female in the territory. Figures in the columns denote sample sizes. Relatedness between deposed female-new female vs. dead female-new female: 0.28 ± 0.27 vs. 0.14 ± 0.19, respectively; *t*-test, $t = -2.48$, df = 67, $P = 0.02$.**

26 of the 28 cases in which the female was deposed, the identity of the dominant male was known before and after the female's status changed. In 18 of these 26 cases (69%) the dominant male remained the same.

SUBORDINATE OR FLOATER

Logistic regression analysis was used to investigate which factors determined whether a deposed female became a demoted subordinate or a floater. The level of relatedness between the old and new females in the territory was excluded from the logistical analysis for two reasons: (1) there was no difference in relatedness for cases in which the female was demoted to a subordinate, as opposed to a floater (demoted-new vs. floater-new; 0.29 ± 0.29 vs. 0.27 ± 0.24; *t*-test, $t = 0.15$, df = 19, $P = 0.88$) and (2) relatedness measures were only available for a subset of the samples and would reduce the power of the analyses considerably. The only factor that remained in the final model was territory quality, whereas the terms female age, previous subordinate experience, the presence of male, or female, subordinates and whether the dominant male was new, had no significant effect (see Table 2c). No significant interactions between the terms in the models were detected. The quality of the territory on which females became demoted subordinates was significantly higher than the quality of the territory from which females became floaters (Fig. 2).

AGE AND TENURE

The logistic regression analysis (Table 2) showed that there was no effect of age on the probability that a dominant female will be deposed or not (5.24 ± 3.10 vs. 5.04 ± 2.96 years, respectively), or whether, after being deposed, a female will become a subordinate or a floater (5.68 ± 3.28 vs. 3.67 ± 1.50 years, respectively). In cases in which the dominant female died the new dominant female was significantly younger than the dead female (3.25 ± 2.13 vs. 5.48 ± 2.95 years, respectively; Wilcoxon's paired test $Z = 4.62$, $n = 48$, $P < 0.001$). There was, however, no significant difference in ages between new dominant females and females that were deposed (4.00 ± 2.49 vs. 5.24 ± 3.00 years, respectively; Wilcoxon paired test $Z = 1.38$, $n = 21$, $P = 0.17$), although the smaller sample sizes mean that the power of this test was relatively low.

The length of tenure of dominant females varied between 1 and 14 years. There was no significant difference between the length of tenure of females that died and those that were demoted (3.91 ± 2.69 ($n = 176$) vs. 4.47 ± 2.70 ($n = 19$) years, respectively; Cox regression $\chi^2 = 2.01$, df =1, $P = 0.16$) but females that became floaters had significantly shorter tenure compared to those that became demoted subordinates (2.11 ± 1.05 ($n = 9$) vs. 4.47 ± 2.70 ($n = 19$) years, respectively; Cox regression $\chi^2 = 9.62$, df = 1, $P = 0.002$). The length of life lived after losing dominant status did not differ between demoted and floater females (1.95 ± 1.56 ($n = 19$) vs. 1.56 ± 0.73 ($n = 9$) years, respectively; Cox regression $\chi^2 = 1.18$, df =1, $P = 0.28$). Overall, the estimated total life span of the birds from the different groups did differ significantly (Cox regression $\chi^2 = 11.75$, df = 2, $P = 0.003$); demoted females that remained as subordinates had a longer life span than either of the other two groups (demoted females = 8.11 ± 4.49 years, floaters = 5.22 ± 2.04 years, dead = 5.23 ± 3.09 years).

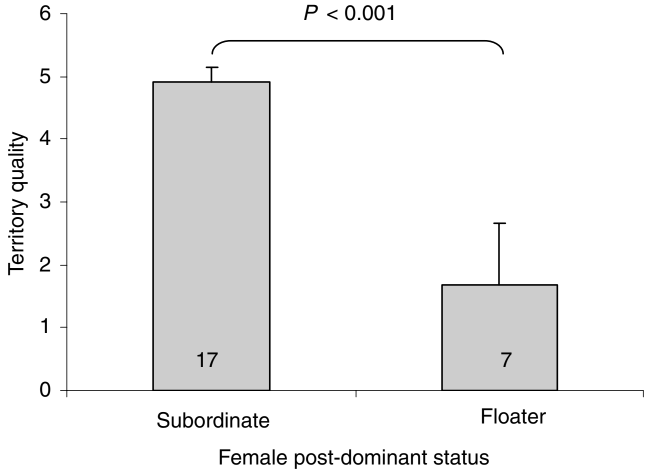

Figure 2. **The quality of the territory inhabited by the dominant female before she lost her position, in relation to her postdominant status. Territory quality was normalized by log transformation. Figures within the columns indicate sample sizes. Territory quality of demoted subordinates versus floaters: 4.91 ± 4.00, $n =$ 17 versus 1.69 ± 0.35, $n = 7$, respectively; unequal variances *t*-test, $t = 3.75$, df = 20, $P < 0.001$.**

RELATEDNESS BETWEEN OLD AND NEW DOMINANT FEMALES

The identity and source of the new female that took over the dominant position was identified in 16 of the 19 cases in which the deposed female became a demoted subordinate. In the other three cases the identity could not be determined, as these birds were not ringed. Nine of the 16 (56%) new females originated from within the same territory as the demoted subordinate, whereas in five cases (31%) the new female was from outside the territory. In two cases, the old dominant female became a subordinate within a new territory after moving there when the dominant male with which she was previously paired moved and set up a new pair bond with the female already resident in that territory.

The distribution of *r* across cases was examined to infer the actual relatedness between the deposed female and the new dominant female. Relatedness varied greatly among individual cases, and showed a similar distribution for both the demoted and floater females (Fig. 3). Females from both categories appeared to be more related to the new breeding female in the territory than one would expect by chance, when *r* would equal zero (demoted–new $r = 0.29 \pm 0.29$, $n = 14$; one-sample *t*-test: $t = 5.32$, df = 47, $P <$ 0.001). However, for floater females, for which there was only

Figure 3. **Pairwise relatedness between deposed females and the new dominant female for females that became demoted subordinates after being deposed (shaded columns) and females that became floaters after being deposed (open columns).**

a small sample, this comparison was not significant (floater-new $r = 0.27 \pm 0.24$, $n = 7$; one-sample t-test: $t = 2.99$, df = 6, $P = 0.24$).

PARENTAGE AND RELATEDNESS TO OFFSPRING

We attempted to determine if demoted subordinate females gained direct parentage by laying eggs in the territorial nest. Unfortunately, the small number of demoted subordinates per year (10% of subordinates on the ca 30% of cooperative territories), and the fact that molecular parentage data were only available for the period 1997–2000, limited the sample of chicks of assigned parentage that hatched while a demoted subordinate female was present in the territory to just eight (from seven independent territories). In none of these cases was the demoted female assigned as the mother. A previous study showed that 26 of 101 offspring (26%) from cooperatively breeding territories were produced through normal subordinate female parentage (Richardson et al. 2002). Although no demoted subordinates were observed to produce offspring, the null expected value was low and there was no detectable difference in the success of normal and demoted subordinates (0/8 vs. 26/101; Fisher's exact test, $P = 0.10$). The relatedness between demoted subordinate females and the offspring produced in their territory was significantly higher than zero (0.24 ± 0.23; one-sample t-test: $t = 2.76$, df = 6, $P = 0.03$).

HELPING BY DEMOTED SUBORDINATES

The nest monitoring provided qualitative data on helping behavior for a total of 14 demoted female subordinates: 13 females were observed during incubation and 11 during provisioning (the two groups did not comprise all the same individual females). In total, 8 of 13 (62%) of demoted females participated in incubating, whereas 7 of 11 (64%) were observed to provision the nest. One female that was not observed incubating was observed provisioning, therefore in total nine of 14 (64%) of the demoted subordinates were observed to assist at the nest in some way (either incubating or provisioning). The reproductive success of the territories containing demoted subordinates was known for 15 of the 19 cases. Of these, only eight successfully produced any offspring. The sample size, and the fact that many of these territories also contained normal prereproductive subordinates (three with two, and five with one), meant that it was impossible to determine whether demoted subordinate helping alone increased the reproductive success of the dominant pair.

Discussion

The present study shows that in the Cousin island population of the cooperatively breeding Seychelles warbler a significant proportion (13.7%) of females that gain a dominant position do not hold onto the territory until their death, but are deposed from their dominant position while still alive. Males, on the other hand, were significantly less likely to be deposed from a dominant position (3% of cases). After being deposed, a large proportion (68%) of these females remained on the same territory as subordinates within cooperative breeding groups, where they helped to raise group offspring. On average, approximately 10% of all subordinates in any year were such demoted female subordinates.

Why are some females deposed from the dominant position? One factor that significantly predicted this was whether a female had been a subordinate before acquiring a dominant position—those that had were much more likely to be deposed. Why this is the case is unclear. One possibility is that females that were subordinate before gaining a dominant position were of lower intrinsic quality than females that directly gained a dominant position, and this difference in quality also determined how likely they were to be deposed. The evidence from other bird species regarding the individual quality of subordinates is equivocal; some studies have provided evidence that stronger offspring prefer to remain as subordinates (Strickland 1991; Ekman et al. 2002) whereas others show that it is the poor-quality individuals, that cannot compete for vacancies elsewhere, that remain (Richner 1990). Further experimental work is required to investigate this in the Seychelles warbler.

Another suggestion was that if dominant females are normally deposed by female subordinates from within their own territory, then dominant females on territories containing female subordinates would be more at risk of being deposed. However, neither the presence nor number of male or female subordinates had any effect on the probability that a dominant female would be deposed. There was also no effect of territory quality or female age.

Relatedness between females that were deposed from a dominant position and those that subsequently took over the position was higher than between those females that died and the females that replaced them. Indeed, the level of relatedness between the deposed female and the new dominant female ($r = 0.28$) was, on average, approximately that expected between second-order relatives (0.25). This result raises the possibility that relatedness itself may play a direct role in whether a female will be deposed, or indeed whether the dominant female may actively step aside to let a related female take over the dominant position. However, as the presence of female subordinates did not increase the chance of a female being deposed and nearly half of the replacements were from outside the group, this does not seem to be the case. It is not just a case of female subordinates within the group waiting to take over from the dominant female.

Why, after being deposed, do some females become demoted subordinates whereas others become floaters? The quality of the territories on which females remained as subordinates was significantly higher than that from which deposed females became floaters. This result concurs with earlier studies that showed that only high-quality territories are able to support the presence of subordinates (Komdeur 1992, 1994b; Komdeur and Edelaar 2001). On a high-quality territory there is little competition for food, so the deposed female can remain as a subordinate, whereas on low-quality territories there would be competition for food and, as a consequence, the female leaves and becomes a floater. The presence of other subordinates did not affect the probability that a deposed female would remain as a subordinate. This is, perhaps, a little surprising as one would have thought that there would be a trade-off between overall group size and territory quality. However, this was also ruled out by the lack of an interaction effect between the number of subordinates and territory quality on the probability that a deposed female remained as a subordinate. None of the other variables, including female age, previous subordinate experience, or the presence of a new dominant male, had any effect on whether the deposed female remained as a subordinate. Finally, deposed females that became subordinates were not more related to the replacement female than were floaters (0.29 and 0.27, respectively), thus ruling out the prediction that deposed females would only remain as demoted subordinates when replaced by a highly related female.

Whether the decision to stay or to float is an active choice by the deposed female is unknown. On low-quality territories the deposed female may leave to reduce competition for food and so increase the reproductive success of relatives left in the group; alternatively the other group members may force her out of the territory. On high-quality territories the demoted subordinate may stay to enhance the reproductive success of the group by helping, or just because she is not forced out. As these “decision” events are extremely rare, and only identifiable after the event, it seems unlikely that we will be able to resolve these questions. However, understanding the potential benefits that could be gained by deposed females remaining as subordinates may lead to a better understanding of the evolution of this behavior.

THE BENEFITS OF BEING A SUBORDINATE

In most cooperatively breeding vertebrates individuals delay dispersal and help their parents raise subsequent offspring; consequently, indirect fitness benefits (kin selection; Hamilton 1964) were suggested to be the primary force driving the evolution of helping (Emlen and Wrege 1989; Mumme et al. 1989; Koenig et al. 1992; Emlen 1997). However, direct benefits have also been shown to be important in many systems (reviewed in Cockburn 1998; Richardson et al. 2002). In the Seychelles warbler, 44% of subordinate females gain direct parentage within a breeding season, and access to direct breeding opportunities appears to be the main driving force behind the evolution of cooperative breeding (Richardson et al. 2002). However, as no cases of demoted subordinate parentage were identified in the present study, it is tempting to suggest that direct breeding benefits may not be important to these individuals, but the small sample size (only eight chicks) means that the possibility cannot be ruled out. Another possibility is that demoted subordinates are better placed to regain a dominant territory. Indeed, three of the 19 (16%) demoted subordinates identified did regain a dominant position, whereas none of the nine floaters did, although sample sizes were too small for a meaningful comparison.

Demoted subordinates helped the dominant pair within the territory by incubating eggs and provisioning nestlings. Although the present study could not measure the number of additional offspring produced as a result of this help, previous work has shown that each subordinate helping on a territory was associated with an increase in the dominant pairs' reproductive output (Richardson et al. 2002). Demoted subordinates were significantly related to the female breeders that they helped ($r = 0.29$). More importantly, they were as related to territory nestlings ($r = 0.24$) as were normal actively helping female subordinates ($r = 0.27$, Richardson et al. 2003a,b) and, consequently, would gain the same level of indirect benefits from helping. Because the role of direct benefits appears to be less important in these demoted subordinates, it seems plausible to suggest that the indirect benefits of helping are driving this particular behavior.

It was, however, obvious that not all demoted female subordinates were highly related to the dominant females that they helped—some were first—or second-order relatives, whereas others were unrelated (see Fig. 3). This variation in relatedness may be explained by a number of reasons: First, even replacement females from within the territory are not always direct offspring of the demoted female because: (1) subordinate females within a territory also produce offspring (Richardson et al. 2001, 2002) and (2) the demoted female may herself have replaced the original female that produced the new dominant female. Second, the new dominant females are sometimes from outside the territory.

The demoted subordinates identified in the present study could be loosely defined as grandparent helpers, as many do appear to be helping their daughters to raise the next generation of offspring. However, the "grandmother hypothesis" (Williams 1957; Hawkes et al. 1998; Lee 2003) specifically refers to the evolution of a prolonged postreproductive stage as a result of senescent females being better able to gain greater fitness by increasing the success of their offspring, rather than continuing to breed themselves. In the Seychelles warbler there was no evidence to suggest that the demoted subordinates were postreproductive, with many other females continuing to breed successfully up to 10 years of age (Komdeur 1996b). It could be argued that, although females are not postreproductive after six years of age, there is a negative effect of age on reproductive output (Komdeur 1996b) and, consequently, older females should step aside to allow their younger kin to breed. Although the mean age at which a female was demoted (5.04) did roughly coincide with the age at which reproductive senescence has been shown to occur in this species (ca six years, Komdeur 1996b), no effect of age on the probability that a dominant female was demoted was detected. Also, the median age at which a female was demoted was only four years, which is below the age at which the number of fledglings produced by females per year peaks in this species (Komdeur 1996b). Furthermore, some females were not demoted until they were 11 years of age. Finally, this hypothesis does not fit with the fact that three of the demoted females later managed to regain dominant positions and attempted to breed again.

Overall, the data from the present study indicate that, as predicted, related individuals normally depose females. However, whether a female remained as a demoted subordinate was not a response to how related the replacement female was to her, but it was determined by whether the territory was of sufficient quality to sustain another subordinate. Demoted subordinates did not appear to be postreproductive and the timing of the loss of dominance was not linked to the onset of reproductive senescence. Consequently, there was no evidence that this was part of a strategy enabling senescent females to gain greater fitness by increasing the success of their offspring, rather than continuing to breed themselves. Instead, it appears that females may be unwillingly deposed from their dominant positions. However, as they are normally replaced by related females—in 57% of cases the new dominant female was identified as being a daughter, sister, or niece—a deposed female can make the best of a bad situation by remaining as a demoted subordinate and gaining indirect benefits by helping to raise related group offspring.

One clear pattern observed was that males were deposed less often than females. Within the Cousin island population, the overall sex ratio is equal (D. S. Richardson, unpubl. data) and more predominant females than males remain as subordinates within their natal territory (Komdeur 1996c; Richardson et al. 2002). Consequently, if anything, one would expect there to be more males competing for dominant positions (and so a higher frequency of dominant males being deposed) than females. However, the high level of extra-pair paternity observed in this species (40%, Richardson et al. 2001) means that relatedness between dominant males and any territory offspring that depose them will, on average, be much lower than for dominant females. Therefore, a reduced ability to gain indirect benefits (because of the lower relatedness) as a subordinate may explain why males are more resistant to being deposed than females. This pattern may be exacerbated if new dominant males are also less willing to tolerate deposed males remaining on the territory as subordinates if, for example, this poses a threat to their paternity.

The presence of older, non prereproductive, individuals that engage in kin-directed cooperative behavior is unusual in the animal kingdom but has been documented, or inferred, in a few mammalian species, for example, in lions, *Panthera leo*, (Pusey and Packer 1994), baboons, *Papio anubis,* (Gouzoules and Gouzoules 1987), pilot whales, *Globicephala macrorhynchus,* (Kasuya and Marsh 1984; Marsh and Kasuya 1984) and humans (Hawkes et al. 1998). Despite the great wealth of studies on cooperative breeding in birds (reviewed in Brown 1987; Cockburn 1998; Koenig and Dickinson 2004), the present study on the Seychelles warbler provides what is, to our knowledge, the first evidence that older, previously dominant individuals regularly become subordinate (grandparent) helpers within such systems. It is plausible that helpers could be the parents of those they helped in other bird species, especially in systems with redirected helping where individuals that lose their own nests become helpers at the nests of kin, for example, long-tailed tits, *Aegithalos caudatus* (Hatchwell et al. 2001) and western bluebirds, *Sialia mexicana* (Dickinson et al. 1996). In such species, all individuals attempt to breed independently each year and only move to helping if their nest fails. Consequently, this behavior would be very different to that of individuals that forgo independent breeding and become subordinate grandparent helpers. The fact that no other studies have reported the occurrence of grandparent helpers may be because it is a rare phenomenon. Alternatively, it could be that the majority of studies on long-lived cooperative breeding bird species, in which grandparent helpers would be likely to evolve, have not yet progressed long enough, or been detailed enough, for such individuals to be identified.

Conclusion

Our results show that in the Seychelles warbler, older, deposed females regularly engage in kin-directed cooperative behavior and thus provide evidence for a new and surprising route to cooperative breeding in birds. However, there was no evidence that this was part of a strategy in which older, senescent females were stepping aside to gain greater fitness benefits by increasing the reproductive success of their offspring, rather than by breeding themselves. Deposed females that remained as subordinate helpers were helping related offspring and could, consequently, gain significant indirect benefits by doing so. Because of this, and the fact that direct benefits appeared to be limited for the deposed females, we suggest that indirect benefits have driven the evolution of such "grandparent" helpers in the Seychelles warbler.

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