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INBREEDING IN THE SEYCHELLES WARBLER: ENVIRONMENT-DEPENDENT MATERNAL EFFECTS

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Abstract.—The deleterious effects of inbreeding can be substantial in wild populations and mechanisms to avoid such matings have evolved in many organisms. In situations where social mate choice is restricted, extrapair paternity may be a strategy used by females to avoid inbreeding and increase offspring heterozygosity. In the cooperatively breeding Seychelles warbler, *Acrocephalus sechellensis*, neither social nor extrapair mate choice was used to avoid inbreeding facultatively, and close inbreeding occurred in approximately 5% of matings. However, a higher frequency of extra-group paternity may be selected for in female subordinates because this did reduce the frequency of mating between close relatives. Inbreeding resulted in reduced individual heterozygosity, which, against expectation, had an almost significant ($P = 0.052$), positive effect on survival. Conversely, low heterozygosity in the genetic mother was linked to reduced offspring survival, and the magnitude of this intergenerational inbreeding depression effect was environment-dependent. Because we controlled for genetic effects and most environmental effects (through the experimental cross-fostering of nestlings), we conclude that the reduced survival was a result of maternal effects. Our results show that inbreeding can have complicated effects even within a genetic bottlenecked population where the ''purging'' of recessive alleles is expected to reduce the effects of inbreeding depression.

Key words.—Environmental variation, heterozygosity, inbreeding avoidance, inbreeding depression, maternal effects, microsatellites, survival.

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Individual genetic diversity, measurable as heterozygosity, is reduced by inbreeding. Low individual heterozygosity may lead to the expression of recessive deleterious alleles and the loss of heterozygote advantage, resulting in decreased reproductive fitness known as inbreeding depression (reviewed in Hartl and Clark 1997; Frankham et al. 2002). Studies have shown a direct relationship between components of fitness and individual genetic diversity (Coulson et al. 1998; Rowe et al. 1999; Slate et al. 2000; Hansson et al. 2001; Hoglund et al. 2002) Inbreeding depression can be substantial in wild populations (reviewed in Crnokrak and Roff 1999; Frankham et al. 2002) and can contribute toward driving populations to extinction (Frankham 1998; Saccheri et al. 1998; Bijlsma et al. 2000; Brook et al. 2002). However, other studies have found no effect of inbreeding (e.g. Keane et al. 1996; Duarte et al. 2003) and considerable debate remains about the importance of inbreeding depression under natural conditions (Lacy 1997; Keller 1998; Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Brook et al. 2002; Keller and Waller 2002).

Various mechanisms have evolved to avoid the possibility of inbreeding in animals, such as, for example, sex-biased dispersal or reproductive suppression (reviewed in Pusey and Wolf 1996). In situations where inbreeding may still occur, that is, where individuals are likely to encounter closely related potential mates, social mate choice to avoid inbreeding should be favored by selection (Crozier 1987; Pusey and Wolf 1996). However, this avoidance behavior does not occur in all species (Craig and Jamieson 1988; Keane et al. 1996; Keller and Arcese 1998) and there are many situations, for example social monogamy, in which social mate choice is restricted and may lead to nonoptimal pairings (e.g. Kruuk et al. 2002).

Extrapair copulations occur in the majority of passerine bird species (Birkhead and Møller 1992; Griffith et al. 2002) and there is evidence that extrapair offspring may be of higher genetic quality (reviewed in Jennions and Petrie 2000; Tregenza and Wedell 2000). This may be due to the acquisition of good paternal genes (Petrie 1994; Hasselquist et al. 1996), or the enhanced genetic compatibility of maternal and paternal genomes (Zeh and Zeh 1996; Brown 1997; Tregenza and Wedell 2000). The use of extrapair matings to avoid inbreeding could be a strategy used by females with restricted social mate choice to increase offspring heterozygosity (Brooker et al. 1990; Bensch et al. 1994) and may explain the widespread prevalence of extrapair paternity (EPP) in socially monogamous species, though the evidence for this so far remains scarce and equivocal (Blomqvist et al. 2002; Foerster et al. 2003).

Selection to avoid inbreeding should be strong within small, restricted populations where there is a high risk of mating with close kin (Frankham 1998). Such populations may be ideal models in which to look for patterns of social and extrapair mate choice. However, it has been suggested that recessive deleterious alleles will be purged in populations that have experienced bottlenecks and/or strong inbreeding in the past (e.g. Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Frankham et al. 2002) and that inbreeding depression may not, therefore, be important in such populations (Caro and Laurenson 1994; Caughley 1994; but see Brook et al. 2002; Reed et al. 2003).

The cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*) is a rare endemic confined to a few small islands within the Seychelles archipelago. By 1959, anthropogenic disturbance had pushed this species to the verge of extinction and only 29 individuals remained on the island of Cousin (29 ha, Crook 1960). The population has since recovered, and has been the focus of intense study since 1985 (Komdeur 1994, 2003; Richardson et al. 2003a,b). 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; 68%, $n = 63$, Richardson et al. 2002). A recent analysis of parentage in the Seychelles warbler showed that joint nesting occurs, with 44% of subordinate females producing offspring and, importantly, that extrapair paternity accounted for 40% of offspring (Richardson et al. 2001).

Our study uses microsatellite markers to genotype individuals and to determine parentage within an isolated population of the Seychelles warbler. We investigate the relationship between mate choice and pairwise relatedness and the consequences of these on offspring heterozygosity and survival. Specifically, we first aim to determine whether social or extrapair mate choice prevents (or reduces) inbreeding. We predict that, compared with primary females, subordinate females will be more related to the primary male within the territory and that they will, therefore, mate with extrapair partners more frequently to avoid inbreeding. Secondly, we assess how often close inbreeding occurs within the population. Finally, we investigate whether there are fitness consequences to inbreeding within this historically small and bottlenecked population.

MATERIALS AND METHODS

Study Population and Data Collection

The entire population of Seychelles warblers on Cousin Island was monitored between 1997 and 2002. During this time, almost all birds (approx. 96%, Richardson et al. 2001) were individually color-ringed and blood-sampled. During the breeding season, each territory was checked for breeding activity at least once every two weeks by following the resident female for 15 min (Komdeur 1992). Nests were observed throughout the breeding cycle and behavioral observations were used (in conjunction with status data from previous years) to determine the status of all birds within each territory. In this study, the primary male and female were defined as the dominant, pair-bonded male and female in a territory, and the term ''subordinate'' included all other birds (>8 months old) resident in the territory. The polymerase chain reaction method devised by Griffiths et al. (1998) was used to determine the sex of each individual.

Birds caught for the first time were ringed with a unique combination of three UV-resistant color rings and a British Trust for Ornithology metal ring. Blood samples (about 15 μl) were collected by brachial venipuncture and then diluted in 800 μl of 100% ethanol in a 2.0-ml screw-cap microfuge tube and stored at room temperature. Dead embryos were extracted from eggs that failed to hatch and stored in 100% ethanol. We attempted to sample all offspring produced in each breeding season. The Seychelles warbler produces one clutch per season and this normally consists of just one egg (80%); however, on occasion two- or three-egg clutches may occur (Komdeur 1991). Many nests could not be reached immediately after hatching and it is possible that mortality occurred in some nests. Therefore, the offspring sampled may not have included all the offspring that hatched, but offspring that were found dead in the egg or nest are included in this analysis. Forty-five offspring (from 40 nests) included in the present study were cross-fostered between nests to separate the environmental and genetic components of offspring survival and fitness. Nestlings of the same hatching date (± two days) were swapped between nests before the sixth day of age (18–20-day nestling period).

Variables including territory quality, the number of offspring and the number of subordinates were recorded for each territory because these parameters may influence parentage and/or survival (Komdeur 1992, 1994, 1996b; Richardson et al. 2001). Because 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 by measuring these variables and calculating territory quality following Komdeur (1994).

Parentage, Relatedness and Heterozygosity

DNA extraction, genotyping, and parentage analysis was completed using CERVUS (Marshall et al. 1998) and the methodology described in Richardson et al. (2001). The simulation program within CERVUS was used to estimate the required critical differences in LOD (the natural logarithm of the likelihood ratio) scores between the first and second most likely candidate parent. Parentage was analyzed for all offspring sampled in 1997–1999 ($n = 199$). Subordinate male parentage did not occur in our dataset and here the term ''extrapair paternity'' (EPP) is used to describe offspring, from either the primary or subordinate female, not fathered by the primary male.

The software program KINSHIP (Goodnight and Queller 1999) was used to calculate individual pairwise relatedness values (r) based on genotypic similarity at 14 microsatellite loci isolated in the Seychelles warbler (Richardson et al. 2000) for: (1) random dyads, (2) primary male-mother dyads, (3) genetic parent dyads, and (4) all combinations of parent-offspring dyads. The mean pairwise relatedness between all random individuals in the population equaled zero. Mean heterozygosity was calculated across the 14 microsatellite loci for each individual.

Inbreeding Avoidance

The pairwise relatedness between a female and her social mate was compared against her mean pairwise relatedness to random males in the population to determine whether females use social mate choice to avoid inbreeding. Female Seychelles warblers may acquire a partner from anywhere on the island through various routes including territory inheritance

(Komdeur and Edelaar 2001a), usurping a territorial female (D. S. Richardson and J. Komdeur, unpubl. data), finding an unattached territorial male (Komdeur 1992), or ‘‘budding off’’ a new territory with a young male (Komdeur and Edelaar 2001b). Consequently, it is impossible to define a specific subset of males that are available to any given female, and using random males probably provides the most accurate estimate of her relatedness to potential mates.

In the analysis of inbreeding avoidance through extrapair mate choice, only cases in which the offspring were assigned with >80% confidence, that is, where the difference in LOD scores between the first and second most likely candidate parent exceeded the simulated critical difference required to be 80% sure of assigning the correct parent (Richardson et al. 2001), were used (n = 185). Nests where the assigned parents had previously been swapped between nests as chicks were also excluded, as these birds could wrongly perceive themselves to be related to the birds within the foster territory ($n = 14$). Although the normal clutch size is one, in some cases a single female may have contributed more than one offspring to the dataset, either because they laid more than one egg in a clutch, or because they produced offspring in more than one breeding season. To avoid pseudoreplication, only one random chick per female per breeding status (a female could be included as both a subordinate breeder and later as a primary breeder; P1 dataset, $n = 97$) was included. However, when comparisons were made between extrapair and within-pair offspring, the dataset included both a within-pair and an extrapair offspring from the same female/breeding status where both were available (P2 dataset, $n = 119$), again selected randomly if there was more than one respective case.

Inbreeding

Apparently close inbreeding, for example between a female subordinate and the male in the territory (often her father), has been observed in Seychelles warblers (D. S. Richardson and J. Komdeur, unpubl. data). However, good pedigree data, where both the paternity of the subordinate and the subordinate’s offspring has been determined using molecular methods, is required to determine directly the frequency of inbreeding. Suitable pedigrees are not yet available in the Seychelles warbler, mainly due to the relatively long life span of this species; maximum life span observed = 21 years (D. S. Richardson and J. Komdeur, unpubl. data) and mean life expectancy = 5.5 years (Komdeur 1992). The frequency of inbreeding was estimated from the distribution of inferred relatedness values between genetic parents (following Duarte et al. 2003). Full sibs should have a symmetrical distribution of relatedness values around an average relatedness of 0.5. Consequently, the proportion of offspring that result from close inbreeding (between first order relatives) can be estimated as twice the number of offspring having a relatedness to their genetic parent exceeding the mean value for first order relatives. The distribution of mother-offspring relatedness values for the P2 dataset was calculated to determine first order relative relatedness. Intraspecific egg dumping has never been observed in the Seychelles warbler (Richardson et al. 2001) and only assigned mothers were used in the analysis.

Survival

The survival of offspring was based on re-sightings of marked individuals during the comprehensive monitoring of the Cousin population, performed for up to six months of each year. Emigration from the island is extremely rare: of 1924 birds, only two (0.10 %) have been observed to fly between islands (Komdeur et al. 2004). Birds that were not re-sighted on Cousin were therefore reliably assumed to have died. Although nestlings leave the nest approximately 16 days after hatching they have a long period of dependence—being fed by parents for at least three months—and remain on the natal territory for at least one year (Komdeur 1992). Consequently survival was measured to one year of age.

We investigated the effect of offspring and parental heterozygosity on survival. The analyses (see statistical details below) were based on the same initial dataset as above ($n = 185$). However, in August 1997, a freak storm during the nestling period resulted in the death of the majority of nestlings from that year. These nestlings were not included in the analyses ($n = 28$). The final dataset ($n = 157$) included cross-fostered offspring, which increased the power of the analyses, but also allowed us to investigate whether any relationship between parental heterozygosity and survival was due to genetic or environmental effects. In the case of cross-fostered offspring, both the allocated social parents differed from the genetic parents, whereas cases of EPP meant that the social father differed from the genetic father.

Terms previously shown to influence survival, that is, territory quality, parental age, the number of subordinates, and clutch size (Komdeur 1992, 1994, 1996b) were included in the initial survival analysis, as were other variables that may have influenced survival, that is, breeding season, mother’s status (primary or subordinate), cross-fostering, and EPP.

Offspring body size may be affected by heterozygosity and may in turn affect offspring survival. Egg size was not measured in this study, but measures of offspring mass and tarsus length were taken. Offspring were measured once, sometime between three days and three months after hatching. Both nestling mass ($F_{1,129} = 141.6$, $P < 0.001$) and tarsus length ($F_{1,123} = 170.7$, $P < 0.001$) had a positive quadratic relationship with the age at which the offspring were measured (days since hatching); consequently the residuals of the functions were taken to obtain a measure of size corrected for age. Body size measurements were only available for two of the offspring that died in the nest, therefore the sample size (and power) for the analysis was much reduced. To avoid reducing the power of the entire survival analysis, body size measurements were only incorporated into a secondary survival analysis based on the minimum model from the initial analysis.

Statistical Analysis

A generalized linear mixed model (GLMM; GENSTAT 1993) was used to test the effects of multiple independent terms, including heterozygosity, on the survival of offspring. The GLMM analysis is a procedure of a generalized linear model with non-normal error structures, which allows both fixed and random terms to be fitted. Random terms control for the effect of repeated measures within a certain unit; for

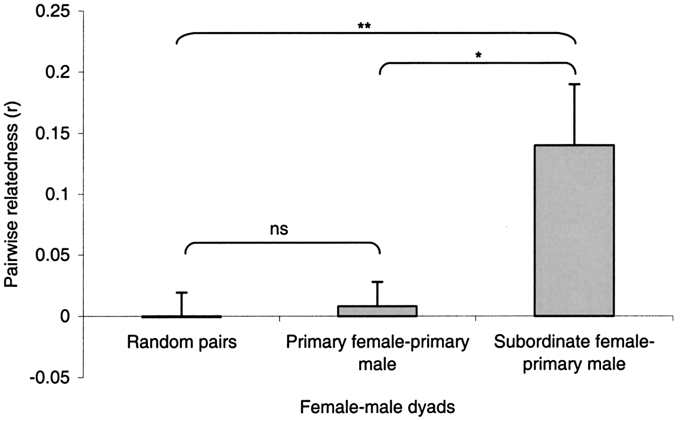

FIG. 1. Mean pairwise relatedness (r) between the mother (either primary female or subordinate) and the primary male in a territory, compared to the pairwise relatedness of random dyads on Cousin Island. Primary female-primary male pairs ($n = 78$) were not more related than expected by chance (0.008 ± 0.22 vs. zero; one-sample t-test; $t = 0.34$, df = 77, $P = 0.73$). Subordinate female-primary male pairs ($n = 19$) were significantly more related than expected by chance ($n = 19$, mean = 0.14 ± 0.20 vs. zero; one-sample t-test; $t = 2.95$, df = 18, $P < 0.01$) and were also more related than were primary female-primary male pairs ($n = 19$ vs. $n = 78$, mean = 0.14 ± 0.20 vs. 0.008 ± 0.22; t-test; $t = 2.33$, df = 95, $P = 0.02$). Error bars represent one standard error. * $P < 0.05$, ** $P < 0.01$.

example, the parent's identity. In the analysis, survival was fitted as the response variate, and the identity of the genetic mother and father were fitted as random effects. All likely explanatory terms were entered into the initial model. The terms breeding season, EPP, female status, territory quality, and cross-fostering were entered as factors. All other terms were entered as variates. Significantly correlated terms were not entered into the final model together as this would lead to a spurious estimation of the variance explained. If two terms, X_1 and X_2, were significantly correlated, X_2 was regressed on X_1, and the residuals from this regression and X_1 were fitted into the model. The data were fitted to a binomial error structure with logit link function and the binomial denominator was set at 1.

In the GLMM the significance of each explanatory term was assessed by its Wald statistic, which is shown along with the level of significance under ''model terms''. The average effects and standard errors of terms that remained in the minimal model ($P < 0.1$), after the sequential backwards removal of nonsignificant terms from the initial model, are shown under ''minimal model''. For terms in the minimal model, statistics and probability values were determined with all other final terms in the model. For terms dropped from the model, values were derived from having all final terms in the model, and then adding each nonsignificant term to the model individually. Interactions are indicated; only the values of significant interactions are presented and their effects are shown graphically using the effects and constant to calculate the predicted means. The significance values of terms contained in the interactions were obtained by running the model without the interactions. Residuals from the minimum model were normally distributed.

All other tests were performed using SPSS 10.7 (SPSS Inc. 1999) and were two-tailed. All data were checked for normality and transformed if necessary before parametric statistical analyses. Unless stated means are given ±SD.

RESULTS

Parentage

Parentage could be assigned with >80% confidence for 185 (93%) of the 199 offspring sampled. Of the assigned offspring, 72 (39%) were extrapair. Using the P1 dataset, a total of 36 of 97 (37%) offspring were extrapair offspring and subordinate females produced significantly more extrapair offspring than did primary females (11/19 [58%] vs. 25/78 [32%]; $\chi^2 = 4.24$, df = 1, $P = 0.04$).

Inbreeding Avoidance

Figure 1 shows the r of the mother-primary male compared to the r of unrelated dyads (zero) on Cousin. For primary females, the mean female-primary male r was not significantly different from the average r of random dyads. However, subordinate females were more related to the primary male than expected by chance and were also more related to the primary male than were primary females (Fig. 1).

Using the larger P2 dataset we found that the mean r between the mother (either primary or subordinate) and the primary male was not significantly different for cases of within-pair offspring compared to extrapair offspring (Table 1). These results did not differ qualitatively when using the smaller P1 dataset. The average r between the mother and the genetic father of extrapair offspring was also not significantly different from that for within-pair offspring for either primary or subordinate females (Table 1). Furthermore, separate analyses using paired tests on the subset of females that

TABLE 1. The relationship between the paternity of offspring and the pairwise relatedness (r) of the mother to (a) the primary male in the territory and (b) the genetic father. Primary and subordinate females are shown separately and together (all). Statistical significance was assessed by t-tests.

	(a) Female-primary male r					(b) Female-genetic father r	
	Within-pair offspring		Extrapair offspring			Extrapair offspring	
Female	n	r_1	n	r_2	r_1 vs. r_2 t	r_3	r_1 vs. r_3 t
All	67	0.04 ± 0.23	52	0.04 ± 0.20	0.02 ns	0.07 ± 0.23	0.57 ns
Primary	58	0.02 ± 0.23	40	0.02 ± 0.20	0.10 ns	0.07 ± 0.23	0.99 ns
Subordinate	9	0.20 ± 0.24	12	0.11 ± 0.20	0.91 ns	0.08 ± 0.23	1.25 ns

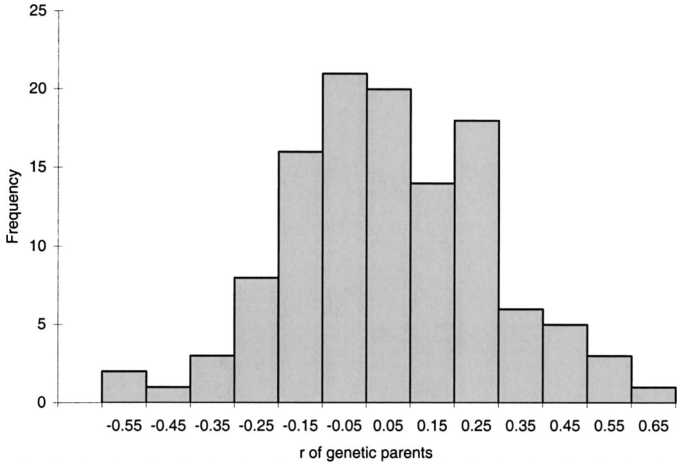

FIG. 2. The pairwise relatedness (r) values of the genetic parents of 119 assigned offspring were distributed around a mean of 0.05 ± 0.23. Mid-points of classes are shown. This distribution displays a right skew and indicates that approximately 5% of matings in the Seychelles warbler population were between close relatives (values centered on 0.46, the value calculated for first-order relatives).

had extrapair offspring also showed that the genetic father of extrapair offspring was not less related to the female than was the primary male in the group (all females; 0.07 ± 0.23 vs. 0.04 ± 0.20; paired t-test, $t = 0.87$, df = 51, $P = 0.39$; primary females; 0.07 ± 0.23 vs. 0.02 ± 0.20; paired t-test, $t = 1.25$, df = 39, $P = 0.22$; or subordinate females 0.08 ± 0.20 vs. 0.11 ± 0.20; paired t-test, $t = 1.13$, df = 11, $P = 0.28$).

Although subordinate females were more related to the primary male than one would expect by chance (see above), those subordinate females that gained extrapair offspring were not more related to the genetic father than expected by chance (0.08 ± 0.23; one-sample t-test; $t = 2.00$, df = 11, $P = 0.32$). Consequently, the extrapair fertilizations gained by subordinate females resulted in offspring that had, on average, unrelated parents.

Inbreeding and Heterozygosity

The estimated r-values between the genetic parents of assigned offspring were normally distributed around a mean of 0.05 ± 0.23 with a range of −0.59–0.61 (test for normality: Kolmogorov-Smirnov $Z = 0.56$, $n = 119$, $P = 0.92$; Fig. 2). The mean r was significantly higher than expected from random within-population matings (one-sample t-test; $t = 2.60$, df = 118, $P = 0.01$), which indicates that inbreeding occurred. Furthermore, the distribution is right-skewed with values exceeding 0.25 in 25 (21%) of cases. Mother-offspring r for assigned offspring was normally distributed around a mean of 0.46 ± 0.17 (range 0.02–0.82; Kolmogorov-Smirnov $Z = 0.89$, $n = 119$, $P = 0.41$), which was significantly lower than the expected value of 0.5 for first order relatives (one-sample t-test; $t = -2.54$, df = 118, $P = 0.01$). A total of five offspring had genetic parents with $r > 0.46$ (see above) and, following Duarte et al. (2003), this would give us an estimated frequency of close inbreeding as 10 matings (twice the number of values exceeding this threshold) or 8.4% of cases. However, our distribution also contained two cases with an r below -0.46. If we assume that these extreme negative cases are artifacts of our rough estimates of r and that two cases may also have occurred on the positive side of the distribution, then our estimate of close inbreeding is reduced to six matings ($(5-2) \times 2 = 6$) or 5% of cases. This estimate is supported by 12 cases in which we had complete data on the parentage of both the offspring and its parents. Of these cases, 1/12 (8.3%) was confirmed as a first-order mating between the subordinate and her father.

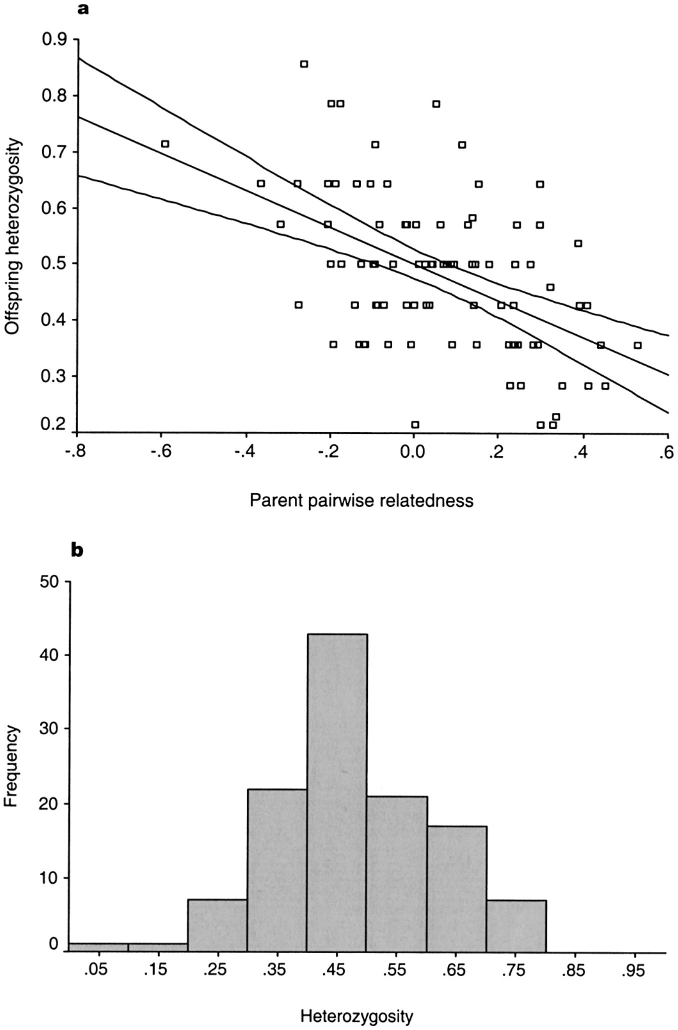

FIG. 3. Heterozygosity of offspring in the Seychelles warbler. (a) There was a negative relationship between the offspring heterozygosity and the parents' pairwise relatedness (n = 97).The regression prediction line with 95% confidence limits is shown ($r^2 = 0.26$). (b) The frequency distribution of offspring heterozygosity ($n = 119$).

There was a negative correlation between the offspring's heterozygosity and the r of an offspring's assigned parents (Pearson correlation coefficient = −0.507, $n = 97$, $P < 0.001$; Fig. 3a). Offspring heterozygosity was normally distributed around a mean of 0.49 ± 0.14 (Fig. 3b; Kolmogorov-Smirnov $Z = 1.18$, $n = 119$, $P = 0.13$). For primary females, there was no difference in the heterozygosity of extrapair or within-pair offspring (0.47 ± 0.13 vs. 0.50 ± 0.14, respectively; t-test; $t = 0.97$, df = 96, $P = 0.33$; Fig. 4). However,

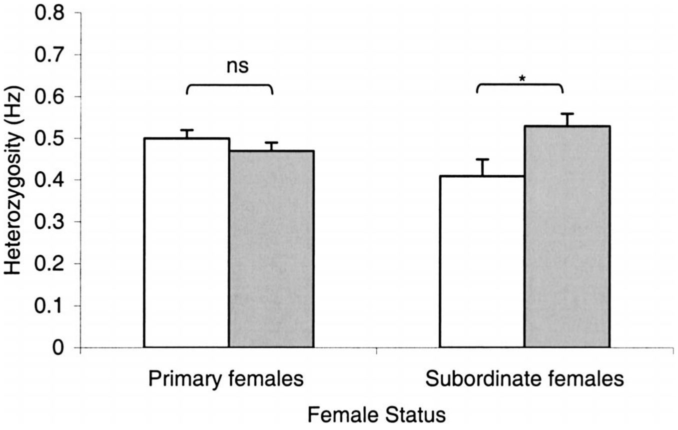

FIG. 4. Heterozygosity of offspring produced by primary and subordinate females. For subordinate females the heterozygosity of within-pair offspring (open columns) was significantly lower than that of extrapair offspring (filled columns) but there was no difference between the two groups for primary females. Error bars represent one standard error. Statistical significance was assessed by *t*-tests. * $P < 0.05$.

for subordinate females extrapair offspring had significantly higher levels of heterozygosity than within-pair offspring (0.53 ± 0.12 vs. 0.41 ± 0.13 respectively; *t*-test; $t = 2.22$, df = 19, $P = 0.039$; Fig. 4). Subordinate within-pair offspring tended to have a lower heterozygosity than primary female within-pair offspring (0.41 ± 0.13 vs. 0.50 ± 0.14 respectively; *t*-test; $t = 1.87$, df = 65, $P = 0.07$). There was no difference in the heterozygosity of extrapair offspring from subordinate or primary females (0.53 ± 0.12 vs. 0.47 ± 0.14 respectively; *t*-test; $t = 1.25$, df = 50, $P = 0.22$).

There was no significant difference between the heterozygosity of the mother and that of the father (0.51 ± 0.13 vs. 0.53 ± 0.14; paired *t*-test; $t = 1.01$, df = 96, $P = 0.31$), nor was there any correlation between the heterozygosities of the two parents (Pearson correlation = 0.04, $n = 97$, $P = 0.73$). Offspring heterozygosity was positively correlated with both the heterozygosity of the mother (Pearson correlation = 0.30, $n = 97$, $P < 0.01$) and that of the father (Pearson correlation = 0.25, $n = 97$, $P < 0.05$).

The Consequences of Inbreeding

Details of the survival analysis are shown in Table 2. Offspring survival was positively related to the genetic mother's heterozygosity and there was also a significant interaction between this and breeding season. Offspring survival was also very close to being significantly negatively related to offspring heterozygosity and was positively related to the heterozygosity of the social father, but not to the genetic father's or the social mother's heterozygosity. Of the other covariates, only the number of subordinates, which had a highly significant positive effect on survival, remained in the final model.

For males, the combined effect of cross-fostering and extrapair offspring meant that the social father and the genetic father were different in 54% (85/157) of cases in the analysis. However, because only 29% (45/157) of the offspring included were cross-fostered, the social and genetic mother was the same for many offspring. Consequently, the environmental and genetic components of the heterozygosity effect were not completely separate in this analysis. To confirm that the social mother's heterozygosity did not affect survival, the genetic mother's heterozygosity was replaced with the social

TABLE 2. Generalized linear mixed model showing the effects of heterozygosity and other terms on offspring survival. The Wald test statistic and level of significance are shown for all terms included in the original model. Terms remaining in the minimum model are highlighted in bold; predicted effect sizes and standard errors are given in the minimal model. Neither the identity of the male nor female constituted significant random terms in the model.

Model terms	Wald statistic	df	*P*
Number of subordinates	**7.90**	**1**	**0.005**
Genetic mother's heterozygosity × breeding season	**9.05**	**3**	**0.029**
Genetic mother's heterozygosity	**3.96**	**1**	**0.047**
Breeding season	**7.83**	**3**	**0.050**
Social father's heterozygosity	**4.71**	**1**	**0.045**
Offspring heterozygosity	**−3.79**	**1**	**0.052**
Genetic father's heterozygosity	2.30	1	0.13
Social mother's heterozygosity	−2.10	1	0.15
Female status	0.70	1	0.40
Cross-fostering	0.44	1	0.51
Territory quality	0.34	2	0.84
Extra-pair paternity	0.01	1	0.94
Clutch size	0.00	1	0.95

Minimal model		Average effect	SE
Constant		0.34	0.26
Number of subordinates		0.81	0.29
Genetic mother's heterozygosity		2.29	2.17
Social father's heterozygosity		3.31	1.65
Offspring heterozygosity		−3.04	1.56
Breeding season	December 1997	0.00	
	July 1998	0.87	0.45–0.76
	January 1999	1.79	
	July 1999	−1.24	

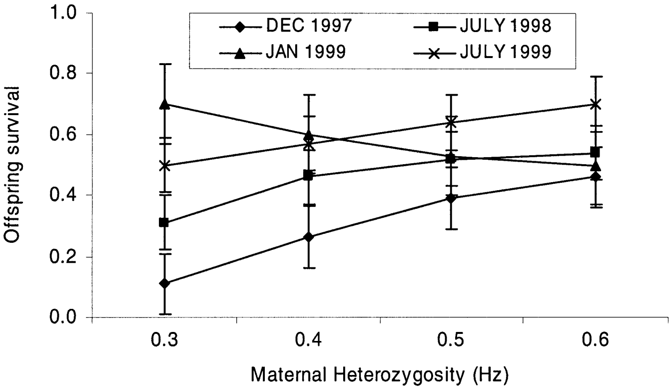

FIG. 5. Relationship between offspring survival and maternal heterozygosity in different breeding seasons. Predicted relationships (means ± SE) estimated from the minimal model (Table 2).

mother's heterozygosity in the final model. The social mother's heterozygosity still had no significant effect and was dropped from the model.

Differences in offspring survival rate between breeding seasons were the basis of the significant interaction between the genetic mother's heterozygosity and breeding season (Fig. 5). In breeding seasons when mean survival was relatively high (i.e., >40%), there was no significant effect of the genetic mother's heterozygosity on offspring survival (July 1998, $\chi^2 = 2.74$, df = 1, $P = 0.098$; July 1999 $\chi^2 = 1.09$, df = 1, $P = 0.30$), or even a trend for a negative effect (January 1999, $\chi^2 = 3.62$, df = 1, $P = 0.057$). In contrast, in the one season when offspring survival was low (i.e., <30%), there was a significant positive relationship between the heterozygosity of the mother and the probability of offspring survival (December 1997, $\chi^2 = 5.98$, df = 1, $P = 0.014$).

Body size measurements from a total of 73 offspring were then incorporated into the survival analysis. Tarsus length and mass were added to the minimum adequate model. Offspring body mass was significantly positively related to survival (Wald statistic = 5.37, df = 1, $P = 0.02$) whereas tarsus length had no effect on the model (Wald statistic = 0.07, df = 1, $P = 0.79$). The rest of the minimum model remained qualitatively the same (offspring body mass, effect size = 0.55 ± 0.24; constant, effect size = 0.64 ± 0.38), and there was no interaction between offspring mass and any measure of heterozygosity.

DISCUSSION

Parentage and Inbreeding Avoidance

Seychelles warblers do not appear to avoid inbreeding through social mate choice, as primary females were no less related to their social mates than expected by chance. A lack of inbreeding avoidance through social mate choice has been recorded in other species, for example, dwarf mongooses, *Helogale parvula* (Keane et al. 1996), song sparrows, *Melospiza melodia* (Keller and Arcese 1998), collared flycatchers, *Ficedula albicollis* (Kruuk et al. 2002), and greater white-toothed shrews, *Crocidura russula* (Duarte et al. 2003), and may reflect the fact that constraints on social mate choice may often occur within animal populations.

Subordinate females were, as expected due to their philopatric nature, more related to the primary male in the territory than expected by chance. In contrast to many other cooperative breeding species, in which the primary female monopolizes breeding (Vehrencamp 2000), subordinate Seychelles warbler females often produce offspring (Richardson et al. 2001). Consequently, subordinate females that mate with the primary male in their own territory will often produce inbred offspring (resulting in the higher than random level of inbreeding observed in this population), though mating with a relative is probably a constraint of being a subordinate rather than due to active mate choice.

The lack of inbreeding avoidance through social mate choice for females creates a situation in which extrapair mate choice may have evolved as a mechanism to avoid inbreeding. Parentage analysis confirmed the results of an earlier study (Richardson et al. 2001) showing that a high level of EPP occurs in the Seychelles warbler (39% of offspring). However, females (primary or subordinate) do not appear to use EPP to avoid inbreeding facultatively, as they did not preferentially gain EPP when closely related to the primary male. Moreover, the genetic fathers of extrapair offspring were no less related to the primary female mothers than were the social fathers. As predicted, female subordinates did have higher levels of EPP than primary females (58% vs. 36% respectively) but, again, this did not seem to be influenced by how related they were to the primary male. Interestingly, although within-pair offspring produced by subordinate females had genetic parents that were more related than expected (inbred), the extrapair offspring produced had parents that were not significantly related.

The analysis of offspring heterozygosity confirmed the parental relatedness results: EPP did not increase the heterozygosity of primary female offspring. However, extrapair offspring from subordinate females were more heterozygous than within-pair offspring (Fig. 4). This can be explained by the fact that subordinate females were often related to the primary group male. Therefore the within-pair offspring of subordinates tended to be inbred, whereas the extrapair offspring had similar levels of heterozygosity to the offspring of the primary females.

Although Seychelles warblers do not appear facultatively to avoid breeding with closely related males, the higher levels of EPP gained by subordinate females can be viewed as an indiscriminate strategy to reduce inbreeding. Overall, subordinate females did produce less inbred offspring as a result of gaining EPP. The higher levels of EPP may be a direct result of other factors; for example, primary males mainly mate-guard the pair female (Komdeur et al. 1999), leaving subordinate females free to pursue, or more exposed to be forced into, extrapair copulations. The lack of mate guarding by the territorial males may be part of an inbreeding avoidance strategy that also benefits the males.

Overall, and in contrast to other bird species where this has been suggested—that is, splendid fairy-wrens (*Malurus splendens*; Brooker et al. 1990), three wader species (Blomqvist et al. 2002; but see Griffith and Montgomerie 2003), and blue-tits (*Parus caeruleus*; Foerster et al. 2003)—primary female Seychelles warblers do not appear to use extrapair paternity to avoid inbreeding and/or increase offspring heterozygosity, though their specific circumstances (living in historically small, restricted populations where there is a high risk of mating with close kin) might have been expected to favor such a strategy. Our result is similar to that obtained in another insular population, of song sparrows (Keller and Arcese 1998), which also found no evidence of inbreeding avoidance in an insular population. However, the higher overall frequency of extrapair paternity gained by subordinate females could be part of an indiscriminate strategy to reduce inbreeding. Further work, reassessing EPP in relation to heterozygosity across a wide range of species, is needed to determine whether the benefit of increased offspring heterozygosity can generally explain the frequent occurrence of EPP in socially monogamous bird species.

Inbreeding

The lack of stringent inbreeding avoidance in the small, restricted, Seychelles warbler population means that inbreeding does occur. Close inbreeding (incestuous mating between first-order relatives) is, according to Ralls et al. (1986), rare in the field ($>2\%$; see also Kruuk et al. 2002), but in the Seychelles warbler we estimated that 5% of all nestlings were the result of such matings. Field observations suggest that this close inbreeding occurs in certain situations; for example, where a female born on a territory has taken over the role of the primary female despite the fact that her father remains the primary male (D. S. Richardson, unpubl. data).

The level of close inbreeding in the Seychelles warbler may actually be higher than we have estimated here, if inbred matings often result in infertile or unhatched eggs. Among birds, close inbreeding has often been shown to affect egg hatchability (Van Noordwijk and Scharloo 1981; Bensch et al. 1994; Westemeier et al. 1998; Kruuk et al. 2002). Unfortunately, the high frequency of EPP, combined with the lack of genotypes, and hence parentage and parental relatedness for unhatched eggs, means that we were unable to measure the effect of inbreeding on hatchability.

Although it is impossible to compare the amount of inbreeding within this Seychelles warbler population with a more outbred population (there are none), our results show that close inbreeding between kin does occur. It is also highly probable that inbreeding has occurred deeper within the pedigree as a result of the small, isolated, and genetically bottlenecked nature of the population. The effect of the genetic bottleneck is apparent in the limited levels of genetic variation seen in the Seychelles warbler (Komdeur et al. 1998; Richardson and Westerdahl 2003).

Heterozygosity and Survival

In the Seychelles warbler, increased relatedness between parents resulted in lower levels of heterozygosity in offspring, hence heterozygosity could be used as a measure of individual inbreeding. Offspring heterozygosity was positively correlated with the heterozygosity of both the mother and father, probably as a consequence of the very limited number of common alleles at the loci examined in the Seychelles warbler (Richardson et al. 2000). In this situation, homozygous parents are likely to be homozygous for the same common allele, therefore offspring are more likely to inherit a different allele from each parent if a parent is heterozygous, than if a parent is homozygous.

Various studies have shown a relationship between individual heterozygosity and survival in the wild (e.g. harbor seals, *Phoca vitulona*, Coltman et al. 1998; red deer, *Cervus elaphus*, Coulson et al. 1999; Arabian oryx, *Oryx leucoryx*, Marshall and Spalton 2000; great reed warblers, *Acrocephalus arundinaceus*, Hansson et al. 2001; and blue tits, Foerster et al. 2003). Surprisingly, our results suggest that increased offspring heterozygosity may have a negative impact on offspring survival ($P = 0.052$). High heterozygosity may be linked to reduced survival in situations where outbreeding depression occurs (reviewed in Templeton 1986; Coulson et al. 1999; Marshall and Spalton 2000; Marr et al. 2002), but this requires crosses between locally adapted populations, which is not the case here.

We do not know why survival appears to decrease with offspring heterozygosity in the Seychelles warbler, but it is a result that is at variance with the enormous body of evidence on inbreeding effects (reviewed in Frankham et al. 2002). One possibility is that this result was an artifact of earlier mortality during the egg phase. Unhealthy inbred offspring with low heterozygosity may never hatch (we were unable to record hatching success in this study), whereas relatively unhealthy non-inbred chicks hatched but later had higher mortality. Further work is required to investigate the validity of this result (a spurious Type 1 error is always possible) and to determine which processes may have caused it.

We also investigated the relationship between the survival of offspring and the parents' level of inbreeding (while controlling for offspring heterozygosity). Offspring from less inbred genetic mothers (high heterozygosity) survived better than did offspring from inbred genetic mothers (low heterozygosity). The heterozygosity of the genetic father had no effect. Therefore, although breeding between closely related Seychelles warblers does not have a direct negative effect on the survival of the offspring, it does result in offspring with low heterozygosity, which, at least for the female offspring, has a subsequent negative effect on their own reproductive success.

Other studies have shown that inbreeding can have a detrimental impact on reproductive success, with reduced reproductive success being linked to low parental heterozygosity (e.g. Meagher et al. 2000; Slate et al. 2000; Amos et al. 2001; Foerster et al. 2003), but in some of these studies the decreased fitness may have been due to the decreased individual heterozygosity of the offspring produced. Our study is, to our knowledge, the first to find an effect of parental heterozygosity on reproductive success after controlling for offspring heterozygosity. However, two other studies, one on the song sparrow (Keller 1998) and one on the takahe, *Porphyrio hochstetteri* (Jamieson et al. 2003), have also shown (using pedigree-based inbreeding coefficients) that reproductive success was detrimentally affected by the mother's, but not the father's, level of inbreeding. Why inbreeding may have sex-specific intergenerational consequences has not yet been determined, but the unequal levels of investment by the sexes in, for example, the egg or nestling appear a likely cause.

In the Seychelles warbler, the effect of the genetic mother's heterozygosity on offspring survival appears to be an inherited environmental maternal effect; that is, an environmental effect on the offspring's phenotype that arises as the product of maternal genes and the maternal environment (Rossiter 1996). One possible explanation could be that mothers with low heterozygosity were of lower quality and provisioned offspring less. However, using the cross-fostering experiment we found no evidence that the foster mother's heterozygosity affected the survival of offspring, thus ruling out the effect of variables in the nestling or fledgling stage. By elimination, our results suggest that early maternal effects, for example, investment in eggs or incubation, caused the positive relationship between the genetic mother's heterozygosity and survival.

Maternal effects may profoundly influence the survival of offspring (Mousseau and Fox 1998; Wolf et al. 1998) and can be important in birds (Price 1998). In the Seychelles warbler, it is possible that mothers with higher heterozygosity are, in some way, of better quality and can invest more in their eggs or in incubation. For instance, more heterozygous mothers may produce larger eggs, which turn into larger chicks, which survive better. However, although offspring mass did help explain survival, the effect of the genetic mother's heterozygosity remained significant and no interaction between the two factors was detected. Alternatively, more heterozygous mothers may be investing more in their eggs in other ways. Studies have shown that differential investment in relation to hormones and immune function components can occur in birds and may affect offspring survival (e.g. Gil et al. 1999; Soler et al. 2003). Indeed in the song sparrow, cell-mediated immunity in chicks, which is thought to be the result of maternal or early environmental effects, is negatively related to the mother's coefficient of inbreeding (Reid et al. 2003) and may explain the sex-specific intergenerational inbreeding effects observed in this species (Keller 1998). Future studies investigating these possibilities in the Seychelles warbler are envisaged.

Offspring survival was positively related to the social father's heterozygosity, but why the social father's, but not the social mother's, heterozygosity affected survival is unknown. The proportion of offspring for whom the genetic and social parent differed was greater for fathers (due to cross-fostering and EPP) than mothers (only due to cross-fostering). Therefore, the analysis will have had more power to detect an effect of the social father's heterozygosity than of the social mother's. However, the difference could be real, with the father's heterozygosity having more effect on the offspring's post-hatching environment than the mother's. For instance, although on average both sexes provision at the same rate in the Seychelles warbler (Richardson et al. 2003a), it is possible that individual rates of provisioning in males may be influenced by their heterozygosity, whereas female rates are not. Further work, exploring the possibility of a link between male heterozygosity and factors that may affect offspring survival, such as provisioning rate and nest-guarding ability, is required.

The interaction between breeding season and the genetic mother's heterozygosity indicates that the magnitude of inbreeding depression was dependent on environmental conditions. In good breeding seasons, when survival was high, there was no effect of the genetic mother's heterozygosity, but in bad breeding seasons, when survival was low, there was a significant relationship. The environmental conditions that affect chick mortality in the Seychelles warbler have not been qualitatively investigated, but variables such as rainfall patterns do appear to affect the chances of breeding attempts being successful (Komdeur 1996c). The results from this study on the Seychelles warbler concur with the evidence from other studies, that has shown that environment-dependent inbreeding depression is important (e.g. Keller et al. 1994; Bijlsma et al. 1999; Keller et al. 2002; Henry et al. 2003). Studies on wild populations must, therefore, monitor the populations over a time frame that encompasses the normal range of environmental conditions experienced by the focal population if they are to assess accurately the impact of inbreeding depression.

It is important to realize, when using neutral molecular markers to measure heterozygosity, that heterozygosity-fitness correlations can occur for different reason. Marker heterozygosity may represent genomewide heterozygosity, or may be due to local effects at particular loci associated with the markers (Hansson and Westerberg 2002). In the Seychelles warbler, heterozygosity did correlate directly with inbreeding and, hence, will reflect genomewide heterozygosity. However, further work is required to investigate whether linkage disequilibrium has resulted in specific markers being associated with linked fitness loci, as appears to be the case in great reed warblers (Hansson et al. 2001).

Inbreeding in Wild Populations

Some biologists have questioned the importance (or existence) of inbreeding depression for the persistence of natural populations (Caro and Laurenson 1994; Caughley 1994), especially in historically inbred populations in which the purging of deleterious recessives may have occurred. However, recent work collectively suggests that purging will not substantially reduce inbreeding depression for most field populations (reviewed in Byers and Waller 1999; Keller and Waller 2002). Moreover, inbreeding effects may also be due to overdominance (although the evidence suggests that this is uncommon), which will continue as long as genetic variation at overdominant loci remains in the population (Charlesworth and Charlesworth 1987). Our results support the view that inbreeding may have deleterious effects within historically small and bottlenecked populations, but also suggests that the link between heterozygosity and fitness can be complicated. Further work is now required to investigate thoroughly the effect of inbreeding upon lifetime reproductive success in the Seychelles warbler.

Conclusion

In the Seychelles warbler, mating between closely related individuals did occur, and neither social nor extrapair mate choice was used facultatively to avoid such inbreeding. However, the offspring of female subordinates—who were often closely related to the dominant group male—were less inbred than expected as a result of the higher levels of EPP gained by subordinate females. Inbreeding resulted in reduced levels of individual genetic diversity (heterozygosity), which, surprisingly, appeared to have a positive effect on offspring survival. Conversely, low heterozygosity in mothers was linked to reduced offspring survival, apparently through maternal effects, though this was an environment-dependent effect. Consequently, for female offspring at least, inbreeding will have a negative effect on the resulting individual's reproductive success later in life. Our results show that inbreeding is not always avoided, even within small isolated populations, and that it can have complicated effects on survival, even after the population has been through a genetic bottleneck in which the ''purging'' of recessive alleles is expected to reduce the effects of inbreeding depression.

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