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Do female great reed warblers seek extra-pair fertilizations to avoid inbreeding?

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Females of many species mate with several males. According to a recent hypothesis, female promiscuity serves to avoid inbreeding. We tested this hypothesis in a polygynous bird, the great reed warbler (*Acrocephalus arundinaceus*), in which extra-pair fertilization, inbreeding and inbreeding depression occur. However, the extra-pair males and social males did not differ in relatedness to the promiscuous females, nor did the least related males sire most of the females' chicks. Thus, contrary to recent findings in some insects, birds and reptiles, we found no evidence for inbreeding avoidance among the promiscuous females. Instead, female great reed warblers may seek other potential benefits when cuckolding, such as good gene effects at particular functional genes.

Keywords: promiscuity; multiple mating; microsatellite; extra-pair fertilization; inbreeding avoidance; relatedness

1. INTRODUCTION

Since the breakthrough of molecular techniques in the late 1980s, it has become evident that females of many species mate with more than one male. It is probable that several processes explain the adaptive significance of female promiscuity, for example reduced male harassment, access to superior foraging sites and paternal care, fertility insurance, inbreeding avoidance and acquisition of 'good genes' (Hrdy 1979; Sheldon 1994; Gray 1997; Petrie & Kempenaers 1998; Tregenza & Wedell 2000). Recent studies on some insects, birds and reptiles suggest that female promiscuity may have evolved primarily to avoid inbreeding and inbreeding depression (e.g. Olsson *et al.* 1996; Blomqvist *et al.* 2002; Tregenza & Wedell 2002; Foerster *et al.* 2003). In sand lizards (*Lazerta agilis*), for example, promiscuous females are mainly fertilized by sperm of less related males, and they thereby achieve enhanced offspring viability (Olsson *et al.* 1996). Similarly, female blue tits (*Parus caeruleus*) select extra-pair partners that increase offspring heterozygosity, a trait that in turn is positively correlated with survival (Foerster *et al.* 2003).

The great reed warbler (*Acrocephalus arundinaceus*) is a socially polygynous passerine bird, in which females

frequently settle to breed in territories of already mated males even though unmated males are available at the breeding site (Bensch & Hasselquist 1992; Hasselquist 1998; Hansson *et al.* 2000a). Males provide most of their parental care to the brood that hatch first on their territory, and substantially less care to later-hatching broods (Bensch & Hasselquist 1994; Sejberg *et al.* 2000). Occasionally, female great reed warblers engage in extra-pair fertilizations (EPFs) thereby raising extra-pair young (EPY; Hasselquist *et al.* 1995). Previous work has shown that the extra-pair males sing with a higher song repertoire than the social male they cuckolded (Hasselquist *et al.* 1996; Hasselquist 1998). We have two lines of evidence that female great reed warblers do not gain direct fitness benefits by mating multiply. Extra-pair males have never been observed to feed their EPY (Hasselquist *et al.* 1996), and male great reed warblers do not seem to commit infanticide as a revenge for non-accepted copulations (Hansson *et al.* 1997).

At Lake Kvismaren, our Swedish study site, male and female great reed warblers are highly philopatric, and as a result many close relatives co-occur (Hansson *et al.* 2002). Among the immigrants, there is a mixture of individuals of different relatedness, mainly because of rare long-distance immigration of females originating from genetically different breeding areas, and also possibly due to population sub-structuring even at relatively small scales (Hansson *et al.* 2003). Thus, genetically related and unrelated individuals breed in close proximity in our study population. Regularly, genetically similar individuals mate and when this happens, they suffer from a significant inbreeding depression in terms of reduced egg hatching success (Bensch *et al.* 1994; Hansson 2003). Furthermore, homozygous offspring have been found to have lower survival prospects than heterozygous ones (Hansson *et al.* 2001).

These genetic and ecological characteristics of the study population make it suitable for examining whether inbreeding is an important factor affecting extra-pair mating patterns. In the present study, we investigate whether promiscuous female great reed warblers preferred extra-pair mates of lower relatedness than their social mates. Furthermore, we test a prediction from the sperm competition hypothesis (Birkhead & Møller 1993), and evaluate if the least related of the males that fertilized the female sire most of her young.

2. METHODS

The great reed warbler is a migratory passerine that winters in tropical Africa. It breeds in reed lakes over large parts of Eurasia. We studied a population of colour-ringed great reed warblers at Lake Kvismaren, southern Central Sweden (59°10' N, 15°25' E). This project was initiated in 1983, and since 1985 we have documented the breeding ecology of all males and females on a daily basis (e.g. Hasselquist 1998). Nests were visited every one to third day, and parameters such as clutch size, number of hatched eggs and fledged young were noted. Chicks were ringed, measured and weighed at an age of 9 days (for details see Hasselquist 1998).

Since 1987, we have collected DNA from all individuals in the population. In broods from the period 1987–1997, we investigated the occurrence of EPY and assigned true paternity to all of these, as well as all of the legitimate young, by evaluating allelic data from either minisatellite DNA-fingerprinting or microsatellite genotyping (Hasselquist *et al.* 1995, 1996; D. Arlt, B. Hansson, S. Bensch, T. von Schantz and D. Hasselquist, unpublished manuscript, available upon request). Concerning the microsatellite analysis, the social fathers could be excluded as genetic fathers by using data from five loci (Aar3-5, G61, Ppi2; D. Arlt, B. Hansson, S. Bensch, T. von Schantz and D. Hasselquist, unpublished manuscript). To assign the extra-pair males, we compared the genotypes of EPY with those of males present in the breeding lake at the time of fertilization. The

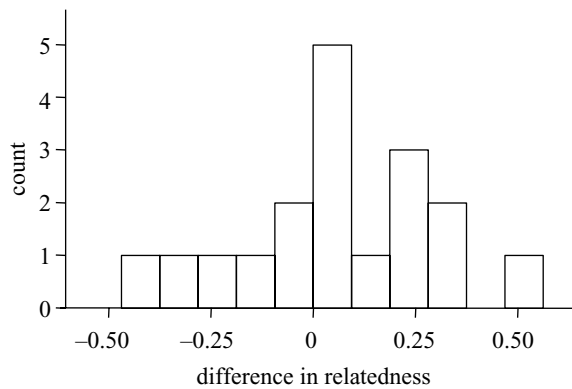


Figure 1. Distribution of differences in relatedness (to the females) between the extra-pair and the social mates in the studied great reed warbler population. Relatedness was based on data from 21 microsatellite loci, and was calculated according to Queller & Goodnight (1989).

relatedness between females and males was calculated from genotype data at 21 microsatellite loci with the program RELATEDNESS 5.0.8 (<http://gsoft.smu.edu/GSoft.html>; Queller & Goodnight 1989). For details concerning DNA-fingerprinting, microsatellite genotyping, parentage analyses and relatedness calculations, see Hasselquist *et al.* (1995, 1996), Hansson (2003), Hansson *et al.* (2000b, 2003) and D. Arlt, B. Hansson, S. Bensch, T. von Schantz and D. Hasselquist (unpublished manuscript).

The association between the proportion of EPY (number of EPY/brood size) and the difference in relatedness (to the female) of the extra-pair and social mates was analysed as generalized linear models in SAS 8.02 (GENMOD procedure with a logistic link function and a binomial error distribution). Because the scale parameter of the models was greater than 1, indicating over-dispersion, we tested the significance of parameters with *F*-tests (dscale option in SAS).

3. RESULTS

Between 1987 and 1997, 18 out of 272 great reed warbler broods (6.6%) contained EPY. Brood sizes ranged from two to six chicks, and the proportion of EPY ranged from 20% to 100%. In each of the 18 broods with EPY there was one extra-pair male, and in all cases the extra-pair male was identified in the study population.

We found no evidence for inbreeding avoidance among the promiscuous females. The extra-pair and the social males did not differ in relatedness to the females (paired test: $t = 1.01$, d.f. = 17, $p = 0.329$). In fact, the average difference in relatedness (to the female) of the extra-pair and the social mates ($R_{\text{difference}} = R_{\text{extra-pair mate}} - R_{\text{social mate}}$) was slightly positive ($R_{\text{difference}} = 0.06 \pm 0.06$ s.e.; figure 1). Furthermore, there was no support for ongoing sperm competition or sperm choice in favour of the less related partner. On the contrary, and against the expectation from such mechanisms, the proportion of EPY in the broods increased slightly with $R_{\text{difference}}$ (estimate of slope parameter = 1.15 ± 1.30 s.e., $F_{1,16} = 0.80$, $p = 0.384$). Females with EPY were somewhat less related to their social males ($R = -0.06 \pm 0.02$ s.e., $n = 18$) than were females without EPY ($R = -0.01 \pm 0.01$ s.e., $n = 228$), but this difference was not significant ($t = 1.55$, d.f. = 244, $p = 0.12$).

4. DISCUSSION

Our results show that female great reed warblers do not avoid inbreeding by soliciting extra-pair matings; (i) the extra-pair males and social males did not differ in

relatedness to the promiscuous females; and (ii) the least related of the two males did not sire most of the females' chicks. Moreover, females with EPY were, if anything, less related to their social males than were females without EPY. Thus, our data contrast the results of some recent studies of insects, birds and reptiles, where females seem to take relatedness into account when mating multiply (e.g. Olsson *et al.* 1996; Blomqvist *et al.* 2002; Tregenza & Wedell 2002; Foerster *et al.* 2003; see the review in Tregenza & Wedell 2000).

However, such contrasting results are not unexpected, but may actually reflect important differences in mating systems and physiology between species (Petrie & Kempenaers 1998). Female great reed warblers are largely unconstrained in their social choice of mates by having the possibility to settle in territories of already mated males (Bensch & Hasselquist 1992; Hasselquist 1998; Hansson *et al.* 2000a). By contrast, sharing territories with other females is a less beneficial option in predominantly socially monogamous species such as the blue tit (Foerster *et al.* 2003). In some populations of blue tits, females have to await vacant territories, and eventually accept the territorial male as the social mate. Therefore, in socially constrained populations of blue tits, extra-pair mating offers an opportunity to avoid fertilizations from related males (Foerster *et al.* 2003). Nevertheless, this reasoning does not apply universally. In sand lizards, for example, females mate multiply despite the fact that they are non-social, thus actually socially unconstrained (Olsson *et al.* 1996). Here, active sperm competition and/or sperm choice in favour of genetically different sperm seem to have evolved. Thus, mating with a close relative has no severe fitness consequences in some species of reptiles, provided that sperm from at least one unrelated male has been received (Olsson *et al.* 1996; cf. Tregenza & Wedell 2002). It should be noted that our analysis did not consider factors such as timing of copulation and amount of sperm, which are known to affect the outcome of sperm competition in birds (Birkhead & Møller 1993).

In conclusion, we have shown that female great reed warblers do not avoid inbreeding by mating multiply. This does not, however, exclude the possibility that other genetic factors play a role for the evolution of female promiscuity in the species. Measuring relatedness may not reflect differences between males at various important functional genes under moderate or strong selection (Reed & Frankham 2001). It was previously found that female great reed warblers engaged in EPY with males singing highly variable song repertoires, and that these males produce the most viable offspring (Hasselquist *et al.* 1996; Hasselquist 1998). Thus, by soliciting copulations from attractive males, female great reed warblers may achieve genetic benefits that are not reflected by the overall genomic variation measured as microsatellite similarity in the present study.

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