Experimental mate replacement does not increase extra-pair paternity in tree swallows

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Before the onset of female fertility, we removed 12 early-settling male tree swallows (*Tachycineta bicolor*) from their nest-boxes and mates, and allowed replacement males which had been floaters to settle with the original female residents. We predicted that females which had their original mate choice altered (experimentals) would be more likely to obtain extra-pair fertilizations than females which remained paired with their original, early-settling mates (controls). The proportion of females obtaining extra-pair fertilizations, however, did not differ between controls and experimentals, indicating that mating tactics of female tree swallows were unaffected by mate replacement. However, differences between early-settling and replacement males did exist. Replacement males had shorter wing chords than early-settling males, suggesting that they were younger. Moreover, a significantly greater proportion of replacement males than early-settling males were unringed and thus new to the study site. Our results suggest that if females are choosing males for good genes, then early-settling males are not superior in genetic quality to the smaller replacement males which had originally been floaters.

Keywords: extra-pair paternity; female choice; good genes; male removals; replacement males; *Tachycineta bicolor*

1. INTRODUCTION

In many species of monogamous passerines, females are known not only to participate in successful extra-pair copulations (Birkhead & Møller 1992), but also to control their occurrence (Smith 1988; Møller 1990; Venier & Robertson 1991; Wetton & Parkin 1991; Houtman 1992; Lifjeld & Robertson 1992; Gray 1996). One explanation for extra-pair matings is that not all males are equal in quality: some may be either genetically or phenotypically 'better' than others, and females choose to copulate with the few high-quality males (Houtman 1992). Male quality has typically been assessed through pairing success, but recent studies have examined potential cues of male quality through nestling paternity (Morton *et al.* 1990; Smith *et al.* 1991; Kempenaers *et al.* 1992; Hill *et al.* 1994; Otter *et al.* 1994; Wetton *et al.* 1995).

Tree swallows (*Tachycineta bicolor*) are well suited to examining male quality and female preferences, because they breed in nest-boxes and are readily accessible for study. They are socially, but not genetically, monogamous: 50–87% of females obtained extra-pair young, resulting in 38–53% of all nestlings being from extra-pair fertilizations (Lifjeld *et al.* 1993; Dunn *et al.* 1994). This high frequency of extra-pair paternity was also observed in a population of tree swallows nesting in natural cavities where 84% of females obtained extra-pair young, resulting in 69% of all offspring being from extra-pair fertilizations (Barber *et al.* 1996). Many females are apparently making mate choices subsequent to pairing.

Tree swallows are obligate secondary cavity nesters that are nest-site limited (Holroyd 1975). Because early arrival is critical in obtaining a breeding opportunity, pressure to be among the first to return to the breeding grounds is intense. Many individuals do not breed in a given year (Stutchbury & Robertson 1987a), but become floaters searching for vacancies.

The purpose of this study was to determine whether a female's initial choice of mate subsequently influenced her mating tactics with respect to seeking extra-pair fertilizations. We removed 12 early-settling males from their nest-boxes and mates, and allowed replacement males (which had initially been floaters) to settle with the resident females. We predicted that control females would be less likely to obtain extra-pair fertilizations than experimental females because their original mate choice was not altered (Møller 1992). We then determined whether males which had obtained and maintained territories early in the season differed morphologically from replacement males. We predicted that replacement males would be smaller than early-settling males because they had been floaters and might therefore be considered as lower-quality males. As replacement males were not among the early settlers, we also predicted that they would be unringed and new to the breeding site, whereas, the early settlers would mainly consist of previously ringed males returning to the study site.

2. MATERIALS AND METHODS

This study was conducted at the Queen's University Biological Station in southeastern Ontario, Canada (44°34' N,

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76°19' W) during the spring and summer of 1993. A population of tree swallows breeds in grids of nest-boxes in the hayfields of the surrounding area (for description of area see Lifjeld *et al.* (1993)). We surveyed 58 nest-boxes daily throughout April to determine settlement patterns by males and females. We then selected 24 of the earliest-settled nest-boxes in early April, and randomly allocated 12 males to the control and 12 to the removal groups. We caught and ringed males and females, and individually marked each one on the wings with unique acrylic-paint codes for identification. Length measurements of the right wing chord (to the nearest 0.1 mm) were taken for control ($n=11$), removal ($n=12$), and replacement males ($n=8$) by one person (C.A.B.) in early May. Control and replacement males had the length of their right tarsus measured (to the nearest 0.1 mm) in mid-June when their nestlings were six days old.

From 6 to 10 May, we removed 12 males by recapturing those which had been designated as removals, took a blood sample (100–150 μ l), and then drove them either 125 or 250 km away before releasing them (Barber & Robertson 1998). Females usually remained at the nest site after their mate had been removed (11 out of 12 females), and accepted the replacement male (10 out of 11 females). Replacement males usually settled within 3 h of the removal, and always within 24 h, concurring with results from another removal study in this species (Lifjeld & Robertson 1992). Laying was synchronous for control and experimental females, because replacement males settled before females became fertile.

(a) DNA fingerprinting

For the paternity component of this study, we collected 100 μ l of blood from the metatarsal vein in six-day-old nestlings, and 100–150 μ l from the brachial vein in adults. To assess the frequency of extra-pair paternity within nests, we fingerprinted 11 control and eight experimental broods. Fingerprinting details, as conducted in the Queen's University Molecular Ecology Laboratory, are discussed in Yezerinac *et al.* (1995) with the only protocol difference being that we digested the DNA with *Hae* III. Both the *per* (Shin *et al.* 1985) and Jeffreys' 33.15 (Jeffreys *et al.* 1985) probes gave identical results in classifying a nestling as within-pair or extra-pair. We therefore combined the bands for both probes and recalculated band-sharing coefficients (*D*) for nestlings (Wetton *et al.* 1987). We scored a mean number of 44.8 ± 1.0 bands per individual ($n=102$ nestlings) for both probes combined (range of 11–63 bands).

The criteria for classifying a nestling as extra-pair were that the nestling must have, for both probes combined: (i) a band-sharing coefficient of less than 0.40 with the social male or female, and (ii) more than four novel bands (cf. Lifjeld *et al.* 1993; Barber *et al.* 1996).

(b) Data analysis

Although 12 male removals were done, we could use the paternity results from only eight nests owing to: (i) the return of two removal males to their nest-boxes; (ii) a female relocating to another nest-box after her male was removed; and (iii) a nest failure. Of the 12 control nests, we had to exclude one from the paternity results owing to nest failure at the egg stage. When testing for familiarity with the breeding grounds, we used data collected on the 12 control males, 12 removal males, and the ten replacement males.

We detected no morphological differences between the control and removal males (early settlers) (two-tailed tests), so we pooled these groups and tested for significant differences

Figure 1. Percentages of extra-pair young within the broods of control and experimental females. Both living and dead nestlings are included.

between early-settling and replacement males. We used one-tailed tests when testing the *a priori* predictions we had made during the designing of this study.

All data were tested for normality by using the Shapiro–Wilk *W* test. Results were corrected for ties, when necessary. All *t*-tests are unpaired unless otherwise specified. Data are means $\pm$ s.e.

3. RESULTS

(a) Control and experimental females

Contrary to our prediction, control and experimental females did not differ in their propensity to obtain extra-pair fertilizations (one-tailed Fisher's exact test: $n=19$, $p=0.66$). Seven out of 11 control females (64%), and five out of eight experimental females (63%) had broods containing extra-pair young (figure 1). Although our sample size is small and the power of our test is therefore low, the direction of our result is counter to that which we had predicted. Therefore, it seems unlikely that a larger sample size would have led to the detection of a significant difference in the predicted direction.

Most experimental females appeared to allow any male to settle: no fights were observed between experimental females and replacement males. However, one female did not allow any male to settle with her after her mate was removed on 10 May, and continuously fought off males (Barber & Robertson 1998). Her original mate successfully homed by 12 May and sired six nestlings with her (no extra-pair young were in the brood). Another female deserted the nest-box after her original mate was removed and discovered a new vacancy at another nest-box, settling there with the resident male. She subsequently had extra-pair young in her brood.

The proportion of females ringed in previous years (which were therefore familiar with the study site) did not differ between the control (4 out of 11) and the experimental groups (4 out of 11) (two-tailed Fisher's exact test: $n=22$, $p=1.0$). The first copulation was observed on 8

May. Experimental females copulated readily with replacement males (we observed six out of the eight pairs copulating, and know that the other two pairs also copulated as confirmed by the presence of within-pair young in their nests). Control and experimental females did not differ in mean laying date (18 May for both, Mann–Whitney U -test: $U=50.5$, $n=12$, 8 , $p=0.85$). The first eggs were laid by a control female on 14 May, and by an experimental female on 15 May.

Out of all the embryos and nestlings, alive and dead, that were fingerprinted, 33.3% (21 out of 63) in the control group and 17.9% (7 out of 39) in the experimental group were extra-pair young. None of the seven extra-pair young from the experimental group was sired by the removal male, indicating that we were successful in removing the males before the females' fertile period began.

There were no instances of intraspecific brood parasitism. All embryos and nestlings were the genetic offspring of the female at the nest, having a mean band-sharing coefficient of 0.61 ± 0.01 ($n=102$) with her. The mean band-sharing coefficient for within-pair nestlings and the genetic father was 0.59 ± 0.01 ($n=74$), whereas that for extra-pair nestlings and the social father was 0.28 ± 0.01 ($n=28$). This latter value is similar to the background band-sharing coefficient found for unrelated adults (the control and experimental pairs) in this population (0.29 ± 0.01 , $n=19$ pairs).

(b) Early- versus late-settling males

Replacement males had significantly shorter wing chords than early-settling males (119.6 ± 1.36 mm compared with 121.5 ± 0.37 mm, $t=1.856$, $n=8$, 23 , $p=0.037$). The percentage of males ringed in previous years (i.e. returning males as opposed to newly ringed males) was significantly greater within the early-settling group (16 out of 24) than within the replacement group (2 out of 10) (66.7% compared with 20%; one-tailed Fisher's exact test: $n=34$, $p=0.017$). However, early settlers and replacement males were similar in mass (21.0 ± 0.25 g compared with 21.6 ± 0.50 g, $t=-1.072$, $n=20$, 5 , $p=0.15$). Tarsus length did not differ between control and replacement males (14.6 ± 0.15 mm compared with 14.4 ± 0.18 mm, Mann–Whitney U -test: $U=54.5$, $n=11$, 8 , $p=0.18$).

4. DISCUSSION

Control females, despite remaining paired with early-settling males with which they had originally settled, were as likely to obtain extra-pair fertilizations as females that became paired with replacement males. Female tree swallows arrive on the breeding grounds after males, and select both the nest site and the male simultaneously (Robertson *et al.* 1992). Our results indicate that females are not exercising final mate choice when they first pair, but are instead settling randomly with regard to males, trying only to secure a breeding opportunity (nest-box and any male). Nestling care in this species is biparental with males usually providing half of the feedings (Leffelaar & Robertson 1986), regardless of nestling paternity (Liffield *et al.* 1993). The choice of which male sires a female's offspring occurs during her fertile period,

when she decides whether or not to engage in extra-pair copulations.

Surprisingly, despite rejecting our hypothesis that mate removal would subsequently affect a female's mating tactics, some expected differences between early-settling and replacement males did exist. Replacement males had significantly shorter wing chords than early-settling males, suggesting that they were younger (Stutchbury & Robertson 1987b). Younger birds might return later from migration. Moreover, a significantly greater proportion of replacement males than control males had never before bred on this study site, and this unfamiliarity could account for their later arrival. Owing to the absence of vacant nest-boxes, later-arriving males become floaters searching for a nesting opportunity. The replacement males were not residents on any of the study sites. Their active intrusions onto several territories in the two weeks prior to their residency affirm that they were floaters, and not males simply upgrading their nesting site.

It may very well be, *post hoc*, that experimental females were the only ones that were able to exercise true mate choice. Given that nesting sites are a limited resource for tree swallows, and that competition for cavities is extremely high, the main priority of females first settling in spring is to obtain a nest-box, any nest-box, regardless of the mate that comes with it. The removal of some females' mates may unexpectedly have given females the ability to choose their subsequent mate. This possibility might explain the lower frequency of extra-pair young within their broods (18% for experimental females compared with 33% for controls), which is opposite to what we had anticipated.

The similar frequency of control and experimental females having extra-pair young in their broods suggests that if females are choosing males on the basis of good genes, then early-settling males are not superior in genetic quality to the smaller replacement males which had originally been floaters. Resident females have been observed engaging in extra-pair copulations with floater males (Barber & Robertson 1999). Females may therefore be assessing males for good genes using cues that are separate from a male's size, familiarity with the breeding site, date of settlement, or even whether he is a resident or a floater. Alternatively, females may be engaging in extra-pair copulations for other genetically based benefits such as genetic compatibility (Brown 1997), genetic diversity of the offspring (Williams 1975), increased sperm competition (Harvey & May 1989; Madsen *et al.* 1992) or to ensure against mate infertility (Gibson & Jewell 1982; Buitron 1983). Although our results do not allow us to reject the good-genes hypothesis, they do indicate that if selection of good genes is the reason for females obtaining extra-pair fertilizations, then early-settling males are not perceived by females as having better genes than floaters.

We thank Laurie Martinovic, Mark Hovorka and Heather Ferguson for assistance in the field, and Laura Santry and Ann Molson for help in the laboratory. We are grateful to the Hughson family for their interest in this project, and to Jessica, Stephanie and Kate Hughson for their visits and help. Logistic support was provided by the Queen's University Biological Station, and the Molecular Ecology Laboratory at Queen's University. Denise Michaud taught C.A.B. the fingerprinting techniques. Mike Gurnsey, Ken Otter, Bart Kempnaers, Kelvin

Conrad, the R.J.R. Bird Lab, Mike Mangel, and four anonymous reviewers made insightful comments and suggestions on previous drafts of this manuscript. Chris Eckert offered statistical advice. Funding was provided by NSERC grants to R.J.R. and P.T.B., and grants to C.A.B. from the North American Bluebird Society, the Animal Behaviour Society, the Frank M. Chapman Fund, the Sigma Xi Scientific Research Society, the Society of Canadian Ornithologists (Taverner award), and the School of Graduate Studies and Research (Queen's University).

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