

High levels of extra-pair paternity in an isolated, low-density, island population of tree swallows (*Tachycineta bicolor*)

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Abstract

Molecular genetic studies have suggested that apparently nonbreeding males ('floaters') may account for a significant proportion of extra-pair paternity (EPP) in avian populations. Attempts to determine the influence of breeding density on EPP are therefore confounded by the presence of a subpopulation of floaters whose numbers are difficult to estimate. To study EPP in a tree swallow (

step in developing such a theory depends upon empirical studies that examine the relationship between the occurrence of extra-pair paternity (EPP) and ecological factors such as operational sex ratio, resource distribution and breeding density (Westneat & Sherman 1997; Petrie & Kempenaers 1998).

Small, isolated populations often have low genetic variation (Frankham 1996) and in species where females seek extra-pair mates for their good genes, EPP may also be low because of the low relative benefits of being choosy (Petrie & Kempenaers 1998; Griffith *et al.* 1999; Griffith 2000). Conversely, high breeding density is expected to increase the potential for extra-pair copulations (EPCs; Møller 1985, 1991) and thus the frequency of EPP (Gibbs *et al.* 1990; Gowaty & Bridges 1991; Westneat & Sherman 1997), because there are more males to perform EPCs and more males from which females may solicit EPCs (Westneat *et al.* 1990; Petrie & Kempenaers 1998). Furthermore, at high breeding densities, nest sites are often limited so the number of unmated floater males (individuals unable to obtain a nest site for breeding; Brown 1969; Stutchbury & Robertson 1985) is greater, providing an even larger pool of extra-pair mates.

While many have predicted a relationship between EPP and breeding density, few empirical studies have been designed to examine the relationship and even fewer have provided evidence for it (but see Bjørnstad & Lifjeld 1997). Dunn *et al.* (1994a) investigated how differences in breeding density and laying synchrony affected EPP by comparing populations of tree swallows (*Tachycineta bicolor*) breeding in nestbox grids and solitary boxes in Onary g3333 TDsing; Bjørnst245 Tc (8(fer)18(encens in br)18(eed-)]TJ inPw 0 0 rg1aasin 7L Ke eedpoppotentis dTJ T* 0 Tw mnmasTc (et al)T,

and a single split-colour band to permit identification of individuals at a distance. For the paternity study, we focused on a section of the nest box grid which contained 32 boxes, of which 21 were occupied at some time during the breeding season. From this area, we obtained blood samples from 13 complete families. We collected 50–100 μL blood samples in 50 μL nonheparinized capillary tubes by brachial venepuncture. Males were sampled while feeding nestlings, to ensure they were social fathers at the nest. We collected nestling blood when the nestlings were 15 days old. We suspended blood in 1 mL of 1 \times

cervus (Marshall *et al.* 1998). Therefore, to confirm the relative proportions of paternities assigned, we performed a second cervus analysis, which we referred to as the 'three-locus run', and compared the populations using only HrU3 and HrU6 from both populations and MS5-29 from Kent Island and Hru7 from the mainland. The parameters input to cervus for both the full-data and three-locus runs appear in Table 1.

Statistical analyses were performed using systat 5.2 (Wilkinson 1992). Means are presented ± 1 SE throughout the text.

Results

Kent Island demographics

The nesting density of tree swallows was lower on Kent Island than in the mainland populations studied previously

(Table 2). During routine mist netting on the island, we rarely caught any swallows that were not breeding in nest boxes and only 40% of the boxes were ever occupied. In contrast, on the mainland grids, about one-third of the birds captured do not breed there and box occupancy always exceeds 80% (R.J. Robertson, unpublished data). During our three-hour removal of males from four nest boxes on Kent Island, only three of the boxes were visited by a total of three tree swallows. All of the visits were brief and none of the visitors remained. In the Ontario population nest visitors are numerous at removal boxes and many of the males removed are permanently replaced by new males within a few hours (Lifjeld & Robertson 1992). In general, the Kent Island population had a much lower density of breeding pairs and floaters.

The degree of breeding synchrony on Kent Island is difficult to assess because comparable data sets for the island and mainland populations (preferably all from the same breeding season) are not available. However, using the range of dates over which clutches were initiated as an index, Kent Island tree swallows started breeding 1–2 weeks later than the mainland box populations (Julian Day 143 vs. 129 and 135; Dunn *et al.* 1994a), but nested for a period intermediate to the values for mainland populations (Table 2).

Microsatellite paternity exclusions

The microsatellite loci were highly polymorphic with the number of alleles per locus varying from five to 43 (Table 3). Most alleles (94%) occurred at a frequency of < 10% and expected heterozygosities ranged from 0.69 to 0.97. None of the loci showed evidence of linkage disequilibrium (all $P > 0.05$) and alleles at all loci were in Hardy–Weinberg equilibrium (all $P > 0.05$) except for those at IBI MS3-13 ($P > 0.001$). T9.270vo(w c5 Tw [or 0 0 0 k rg -87.1])TJ TD 0 Tc

alleles (Pemberton *et al.* 1995) at this locus. The high frequency of null alleles at IBI MS3-13 does not preclude its use for exclusions but we did regard any individual apparently homozygous at IBI MS3-13 as a potential null heterozygote. All exclusions suggested by homozygous IBI MS3-13 were confirmed by at least two other loci, meaning apparent homozygotes were effectively not used for exclusions. The combined exclusion probability of the four loci was nearly 100% (Table 3) and was still greater than 99% (0.995) when IBI MS3-13 was excluded from the calculation. We found no evidence of intraspecific brood parasitism ('egg-dumping'). However, we were able to exclude the putative fathers of 37 nestlings (55%) from 11 nests (85%). The proportion of nestlings that were EPY ($\chi^2_1 = 0.35$, $P = 0.56$) and the proportion of broods containing EPY ($\chi^2_1 = 0.64$, $P = 0.42$) did not differ significantly between Kent Island and the totals of the mainland

$P = 0.75$). In the three-locus run, 20 paternities were assigned on Kent Island (54%) and 35 were assigned on the mainland (60%). Again, there was no significant difference in paternities assigned (χ^2_{12}

experimental manipulations or studies of populations breeding under extreme conditions may be needed to discover significant deviations from the pattern. Despite being one of the most heavily studied avian species, tree swallows are proving to be an interesting challenge for avian mating system theory.

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