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 (*Tachycineta bicolor*) population with few floaters, we chose a nestbox grid on an island with an excess of available breeding sites and very few floaters. We obtained DNA samples from 13 complete families and performed DNA profiling on them using four microsatellite loci. For comparison, we also obtained a sample of 58 extra-pair young (EPY) from a mainland population typed at five microsatellite loci. Paternity assignments among resident males in both populations were made using the microsatellite profiles and a likelihood-based statistical method. Of the 67 island nestlings typed, we found 37 (55%) nestlings from 11 (85%) different nests that were EPY. The proportion of nestlings that were EPY and the proportion of broods containing EPY did not differ significantly between island and mainland populations studied previously. There was no significant difference between island and mainland populations in the proportion of extra-pair paternities assigned among neighbouring resident males. Male breeding density does not appear to affect the ability of female tree swallows to obtain extra-pair fertilizations, at least over the range of densities studied so far. The rate of EPP has remained remarkably consistent over many years, studies and populations implying an important role of active female choice in determining EPP.

Keywords: islands, mating system, microsatellites, paternity analysis, Tachycineta bicolor, tree swallows

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Introduction

In the traditional view of mating systems, the 'environmental potential for polygamy' provided a link between the general ecology of a bird species and its mating behaviour (Emlen & Oring 1977). However, molecular techniques have shown that paternity in many birds does not reflect their social behaviour and that quite different individual mating patterns (*sensu* Ahnesjö *et al.* 1993) may underlie the overt social mating system (Birkhead & Møller 1992; Petrie & Kempenaers 1998). A unified body of theory describing the relationship between ecological factors and mating patterns in the light of data from molecular studies has yet to be formulated, although progress is being made as more studies accumulate (Westneat *et al.* 1990; Petrie & Kempenaers 1998; Griffith 2000). The first

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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 Ontario and Alberta, Canada. Finding no difference in EPP frequency between pairs nesting solitarily vs. in grids or between the breeding locations, Dunn *et al.* (1994a) (see also Kempenaers *et al.* 1999) concluded that breeding density did not affect EPP because EPCs were largely determined by active female choice of extra-pair partners. Furthermore, in Ontario, at least three-quarters of extra-pair young (EPY) in the grid populations were not sired by neighbouring males on the grid (Lifjeld *et al.* 1993; Dunn *et al.* 1994b; Kempenaers *et al.* 1999). Most EPY, therefore, may be sired by floater males (Barber & Robertson 1999; Kempenaers *et al.* 1999).

How far individual floaters may range is not known, but given the mobility of tree swallows (Robertson *et al.* 1992), flights between the grids and solitary boxes studied by Dunn *et al.* (1994a) and Kempenaers *et al.* (1999) are not unexpected. The density of potential extra-pair mates around solitary boxes was still probably lower than on the box grids. However, the presence of an unknown number of floaters means that the density of known breeding males will always underestimate the number of potential extra-pair mates, at least over a scale of a few kilometres. Determining the relationship between breeding male density and EPP in the presence of floaters is therefore difficult unless the site is isolated from other breeding areas (Ewen *et al.* 1999).

To study EPP in a tree swallow population with few floaters, we chose a nestbox grid on an isolated island with a low population density and an excess of available breeding sites. We predicted that in the island population of tree swallows we studied, frequencies of EPP would be lower than in the mainland populations studied previously. We also attempted to assign paternity to EPY, expecting to find a greater proportion of the extra-pair sires in the island population because of the lower breeding density and low proportion of unsampled, floater males. This is the first study to consider the influence of floater density on EPP frequency.

Materials and methods

Field methods

We performed our field study between 30 May and 21 July 1993 at the Bowdoin Scientific Station on Kent Island, an 80-ha island, near New Brunswick, Canada (44°35′ N, 66°46′ W). Kent Island is part of the Three Island Archipelago, the total area of which is 127 ha. Tree swallows have been observed to breed only in boxes on Kent Island in the archipelago. Few, if any natural cavities exist on the islands because woodpeckers do not breed there. The nearest suitable nesting habitat is 9 km away on Grand Manan Island and 20 km away on the coasts of Maine and Nova Scotia (Wheelwright *et al.* 1991).

One hundred and thirty-four nest boxes were placed in a grid with 30 m between boxes along a column or row, over an area of about 8 ha. The population has been studied since the nest boxes were first established in the 1940s (Paynter 1954). Over the 7 years prior to our study the population has been declining (N. Wheelwright unpublished data). In 1993, when the study was conducted, only 54 (40%) of the available breeding sites were occupied.

To gauge the density of floater males in the population, we recorded the identity and behaviour of all tree swallows visiting the nest boxes and we used mist nets placed throughout the colony to sample flying birds. We also temporarily removed four established males during days 2–4 of egg-laying and held them in captivity for 3 h while we monitored the nest boxes for intrusions and possible replacement males.

Blood sample collection and storage

We attempted to catch every adult tree swallow on Kent Island and determine where it nested. We banded all birds with US Fish and Wildlife Service aluminium bands

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× Applied Biosystems Lysis Buffer and stored it at 4 °C until we extracted the DNA according to Lifjeld *et al*. (1993). Extraction and subsequent genetic profiling were performed at the Queen's University Molecular Ecology Laboratory (QUMEL).

Microsatellite paternity exclusion

We used four loci for microsatellite profiling: IBI MS3-13, IBI MS5-29, HrU3 and HrU6. IBI MS3-13 and IBI MS5-29 were isolated previously from a tree swallow genomic library (Crossman 1996). HrU3 and HrU6 were previously isolated from barn swallows (*Hirundo rustica*; Primmer *et al*. 1995) and are known to be useful for typing tree swallow samples (Kempenaers *et al*. 1999). Microsatellite loci were amplified from genomic DNA by polymerase chain reaction (PCR) using primers end-labelled with [α^{33}P]-dATP. PCR products were arranged into family groups and run out on a 5% polyacrylamide denaturing gel with a sequenced size standard. Dried gels were exposed to X-ray film overnight to produce autoradiographs for scoring. Complete details of this methodology appear in Crossman (1996) and Kempenaers *et al*. (1999).

Microsatellite alleles are assumed to be inherited in simple Mendelian fashion and parentage was assessed by simple exclusion. Any allele amplified at any locus not corresponding to an allele amplified in either the putative mother or father was taken to indicate an EPY. All exclusions were supported by at least two loci.

Basic population genetic statistics were generated with the computer programs POPGENE 1.21 (Yeh & Boyle 1997) and CERVUS 1.0 (Marshall *et al*. 1998). The data were tested for deviations from Hardy–Weinberg equilibrium at each locus and linkage disequilibrium at each pair of loci by the Markov chain methods in GENEPOP 3.1 (Raymond & Rousset 1995).

Paternity assignment

Paternity assignment was performed using the microsatellite profiles and the likelihood-based method in the computer program CERVUS 1.0 (Marshall *et al*. 1998). CERVUS calculates an LOD score as the natural logarithm of the product across all loci of likelihood ratios comparing a potential father to an individual drawn from the population at random (Meagher 1986; Marshall *et al*. 1998). The male with the highest positive LOD score is the most likely father of an offspring. The statistical confidence of the LOD score is assessed as Δ, the difference in LOD between the two most likely fathers (Marshall *et al*. 1998). Critical values for Δ are generated in CERVUS by bootstrapping from the experimental population (Marshall *et al*. 1998). CERVUS takes into account typing errors arising from the presence of null alleles and limits the number of extra-pair paternities according to the estimated proportion of potential fathers that have been sampled (Marshall *et al*. 1998).

To compare the success of paternity assignment with a mainland population, we obtained a sample of 20 families from a grid of nest boxes ('Jeremy's Grid') at the Queen's University Biological Station (44°34′ N, 76°20′ W; Mitchell & Robertson 1996; Kempenaers *et al*. 1999). We chose Jeremy's grid as a nestbox grid structurally and spatially similar to our study site on Kent Island, but with high potential for intrusions by floaters or resident birds from neighbouring grids and nearby natural cavity populations. Jeremy's grid is a single nestbox grid subset from the data presented in Kempenaers *et al*. (1999), which included a number of nestbox grids, clusters and solitary boxes from a wider area. The Jeremy's grid subset consisted of 101 nestlings containing 58 EPY. All nestlings and their putative parents had been typed previously at five microsatellite loci (Hru3, Hru5, Hru6, Hru7, IBI MS3-31; Kempenaers *et al*. 1999).

Our goal in assigning paternities in the two populations was not to estimate the number of extra-pair fathers present but instead was to make a reasonably equitable comparison between the populations of the maximum potential number of paternities that could be assigned to neighbouring males. To this end, we used CERVUS differently than if we were simply seeking to assign paternities. First, for our critical values, we chose values of Δ that provided '80% confident paternities' (Marshall *et al*. 1998). Our principal concern was to find the maximum number of potential extra-pair fathers, and this level of confidence provides a reasonable compromise of power and certainty, given the relatively small size of our samples. Second, for the pool of potential fathers, we included only males resident on the grids (13 males for Kent Island, 19 for the mainland) and assumed that all potential fathers had been sampled in both populations. Although this treats the data as if there were no floaters in either population and is therefore unrealistic, it provides a means of comparing the maximum potential number of paternities assignable within our known samples. Finally, the high frequency of null alleles we found at one of the loci we used for typing Kent Island might have biased paternity assignment using

Table 1 Parameters used in CERVUS 1.0 (Marshall *et al.* 1998) for maximum likelihood paternity assignment. The full-data run used four microsatellite loci from Kent Island and five loci from the mainland (Table 3). The three-locus run used HuU3, HrU6 and IBI MS5-29 from Kent Island and HuU3, HrU6 and HrU7 from the mainland (see text)

	Mainland	Kent Island
Full-data run		
Simulation cycles*	10 000	10 000
Proportion of candidate parents sampled†	1.00	1.00
Proportion of loci typed‡	0.83	0.92
Proportion of loci mistyped§	0.0121	0.0693
Strict confidence level¶	80%	80%
Expected paternities assigned**	79 (78%)	51 (77%)
Observed paternities assigned**	68 (67%)	48 (72%)
Three-locus run		
Simulation cycles*	10 000	10 000
Proportion of candidate parents sampled†	1.00	1.00
Proportion of loci typed‡	0.96	0.97
Proportion of loci mistyped§	0.006	0.006
Strict confidence level¶	80%	80%
Expected paternities assigned**	93 (92%)	58 (86%)
Observed paternities assigned**	78 (77%)	50 (75%)

*Number of bootstrap simulation cycles used to estimate critical LOD values.
†All extra-pair fathers were assumed to be among the sample of neighbouring males to maximize the number of paternities assigned in both populations (see text).
‡Calculated by CERVUS 1.0 (Marshall *et al.* 1998).
§Mean of the CERVUS estimates of null allele frequencies across all loci.
¶Selected to favour assignment of extra-pair paternities to neighbouring males in each population (see text).
**Includes Non-EPY.

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 Comparison of breeding conditions between Kent Island and mainland populations of tree swallows

	Kent Island	Mainland*
Breeding synchrony	24 days	21 and 30 days†
Population	Isolated	Continuous with others
Proportion of boxes occupied	40%	> 80%
Floater density	Rarely observed	High
Nesting density (pairs/ha)	6.5	16.1

*Dunn *et al.* (1994a,b).
†Estimated as the days between the earliest and latest first egg dates.

(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 ($P > 0.001$). The low heterozygosity of IBI MS3-13 (Table 3) appears to result from a high number ($n = 16$, estimated by CERVUS, Marshall *et al.* 1998) of probable null

Table 3 Characteristics of microsatellite loci used for paternity exclusion and assignment

Locus	n	Alleles	H_O	H_E*	P_{EXCL}†	F_{IS}
Kent Island population						
IBI MS3-13	63	23	0.49	0.94	0.860	0.472
IBI MS5-29	92	5	0.73	0.69	0.450	− 0.050
HrU3	89	29	0.96	0.95	0.900	− 0.006
HrU6	93	43	0.96	0.97	0.920	0.003
Mean/Total		84	25	0.78	0.89	0.999
Mainland population						
HrU6	136	43	0.92	0.92	0.830	0.019
HrU7	140	7	0.70	0.72	0.504	0.036
HrU3	138	29	0.93	0.94	0.880	0.004
HrU5	135	35	0.97	0.94	0.883	− 0.050
IBI MS3-31	26	8	0.92	0.85	0.664	− 0.136
Mean/Total		115	24	0.89	0.88	0.999

*Nei's (1987) expected heterozygosity.
†Probability of exclusion from Chakravarti & Li (1983) and Marshall *et al.* (1998).

Table 4 Comparison of rates of extra-pair paternity between Kent Island and mainland box grid populations of tree swallows

Source	Study year	Nestlings that are EPY	% Nestlings that are EPY (95% CI)	Broods with EPY	% Broods with EPY (95% CI)
Mainland Total	1990–95	272/529	51 (47–56)	79/106	74 (66–83)
Dunn *et al.* (1994a)*	1990	92/181	51 (44–58)	23/34	68 (52–83)
Dunn *et al.* (1994b)	1991	63/119	53 (44–62)	20/23	87 (73–100)
Kempenaers *et al.* (1999)†	1995	117/229	51 (45–58)	36/49	74 (61–86)
Kent Island	1993	37/67	55 (43–67)	11/13	85 (65–100)
Total nestlings		309/596	52 (48–56)	90/119	76 (68–83)

*Lifjeld *et al.* 1993 is subsumed by Dunn *et al.* 1994a.
†Includes the single nestbox grid, 'Jeremy's Grid' used in this study.

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 ($\chi_1^2 = 0.35$, $P = 0.56$) and the proportion of broods containing EPY ($\chi_1^2 = 0.64$, $P = 0.42$) did not differ significantly between Kent Island and the totals of the mainland populations studied previously (Table 4).

Assignment of paternity

The microsatellite loci used for typing the mainland population were also highly polymorphic with the number of alleles per locus varying from seven to 43 and expected heterozygosities ranging from 0.72 to 0.94 (Table 3). None of the loci deviated significantly from Hardy–Weinberg equilibrium ($P > 0.05$). F_{IS} values in both populations, except for those of IBI MS3-13, were all near zero and heterozygosities in both populations fell into a similar, high range (Table 3), suggesting that both Kent Island and the mainland population support high levels of genetic diversity.

CERVUS assigned 80% confident paternities among resident males to 49% (18/37) of the EPY from Kent Island and 43% (25/58) of the EPY from the mainland in the full-data run. There was no significant difference between populations in the proportion of paternities assigned ($\chi_1^2 = 0.10$,

$P = 0.75$). 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 ($\chi_1^2 = 0.37$, $P = 0.54$).

Discussion

The high vacancy rate of nest boxes on Kent Island, the considerable distance over open ocean to the nearest alternative breeding sites and the scarcity of nonbreeding birds seen or captured during extensive mist netting during the egg-laying period, suggest that there are few floaters and few short-term migrants from nearby populations. In contrast to work in the Ontario population (Lifjeld & Robertson 1992), our removal of resident males revealed relatively few visitors and those visitors that there were intruded only briefly. Taken together, these observations indicate that the density of both breeding and floater males is much lower on Kent Island than on the mainland.

Although a small island population, Kent Island is not sufficiently isolated from the mainland to experience reduced genetic diversity, which is confirmed by the high levels of heterozygosity and low F_{IS} found (Table 3; see also Griffith *et al.* 1999). The distance to the nearest breeding populations is still quite far for a regular exchange of individuals, as might occur with groups of floaters moving between local populations on the mainland. The high levels of heterozygosity and low F_{IS} found in both populations suggest that inbreeding is very rare and members of the breeding population share low relatedness at both sites. High genetic diversity in both the island and mainland populations also means the influence, if any, of genetic diversity on female choice of extra-pair partners would be similar in both populations (Petrie & Kempenaers 1998).

The proportion of Kent Island nestlings that were EPY (55%) and the proportion of nests that contained EPY (85%) were remarkably similar to previous studies of mainland populations (Table 4). In fact, considering that studies of EPP in tree swallows have been carried out over a number of years, in a number of different populations, by a number of different researchers, using a number of different exclusion techniques, the proportion of nestlings that are EPY, and the proportion of broods containing EPY, has remained remarkably consistent (Table 4).

In paternity assignment, CERVUS provides a facility to adjust its statistics for the estimated proportion of potential fathers sampled and is sensitive to variation in that proportion (Marshall *et al.* 1998). The presence of floaters and, in the mainland population, uncounted males breeding in natural cavities and other unsampled males breeding in nearby boxes, means the proportion of unsampled potential fathers cannot be estimated with any accuracy. In order to make a reasonable comparison between the island and mainland populations, we attempted to assign paternity only among the parents we had sampled, and assumed that all potential fathers had been sampled in both populations. The remaining parameters input to CERVUS were calculated from the actual data. Therefore, we have estimated the maximum possible number of EPY that could be assigned to resident males by CERVUS we had sampled, based only on parameters estimated or calculated from the data. A more realistic estimate from more complete data sets for both populations would probably assign fewer extra-pair paternities to resident males. The values determined here represent theoretical maxima and should only be compared with each other.

In three studies that have attempted to assign paternity to EPY, about one-fifth of biological fathers have been found (Lifjeld *et al.* 1993; Dunn *et al.* 1994b; Kempenaers *et al.* 1999). Given the lower breeding density and reduced number of floaters on Kent Island, we expected to find a far greater proportion of extra-pair fathers among the resident males than on the mainland. However, the proportion of EPY assigned paternity by CERVUS did not differ significantly between the island and mainland populations, either in the full-data or three-locus runs, indicating that a similar proportion of extra-pair fertilizations may be attributed to the sampled neighbouring males in both populations. Relatively intensive examinations of resident, neighbouring males in previous studies have also failed to reveal a significant proportion of extra-pair fathers (Lifjeld *et al.* 1993; Dunn *et al.* 1994b; Kempenaers *et al.* 1999), which suggests that the extra-pair fathers may be part of the floater population (Barber & Robertson 1999; Kempenaers *et al.* 1999). On Kent Island, the majority of EPY must have been sired by the remaining 40, unsampled, breeding males on the island.

Dunn *et al.* (1994a) found no effect of breeding synchrony or breeding density on EPY rates in two mainland box populations of tree swallows. Two studies of numerous characteristics usually associated with male quality have failed to find any relationship with paternity success, suggesting that 'good genes' models may not play an important role (Dunn *et al.* 1994b; Kempenaers *et al.* 1999). Our study has failed to find an effect of floater density. The rate of EPP has remained relatively constant across many studies, implying an important role of active female choice (Dunn *et al.* 1994a; Kempenaers *et al.* 1999) and suggesting the level of extra-pair paternity might indeed be characteristic of the species (Owens & Hartley 1998).

A more intensive study of an isolated population such as Kent Island, in which DNA is sampled from every individual seen on the island during the breeding season may prove to be the best opportunity to understand tree swallow mating dynamics. Only in an isolated population can a very high proportion of potential fathers be sampled (Ewen *et al.* 1999). However, given the consistency of EPP across studies and populations of tree swallows, extreme

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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References

Ahnesjö I, Vincent A, Alatalo R, Halliday T, Sutherland WJ (1993) The role of females in influencing mating patterns. *Behavioral Ecology*, **4**, 187–189.

Barber CA, Robertson RJ (1999) Floater males gain extra-pair copulations with resident female tree swallows. *Auk*, **116**, 264–269.

Birkhead TR, Møller AP (1992) *Sperm Competition in Birds: Evolutionary Causes and Consequences*. Academic Press, London.

Bjørnstad G, Lifjeld JT (1997) High frequency of extra-pair paternity in a dense and synchronous population of willow warblers *Phylloscopus trochilus*. *Journal of Avian Biology*, **28**, 319–324.

Brown JL (1969) Territorial behavior and population regulation in birds. *Wilson Bulletin*, **81**, 293–329.

Chakravarti A, Li CC (1983) The effect of linkage on paternity calculations. In: *Inclusion Probabilities in Parentage Testing* (ed. Walker RH), pp. 411–422. American Association of Blood Banks, Arlington, Virginia.

Crossman CC (1996) *Single locus DNA profiling in the tree swallow* Tachycineta bicolor: *a comparison of methods*. MSc Thesis. Queen's University, Kingston Ontario, Canada.

Dunn PO, Whittingham LA, Lifjeld JT, Robertson RJ, Boag PT (1994a) Effects of breeding density, synchrony, and experience on extrapair paternity in tree swallows. *Behavioral Ecology*, **5**, 123–129.

Dunn PO, Robertson RJ, Michaud-Freeman D, Boag PT (1994b) Extra-pair paternity in tree swallows: Why do females mate with more than one male? *Behavioural Ecology and Sociobiology*, **35**, 273–281.

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215–223.

Ewen JG, Armstrong DP, Lambert DM (1999) Floater males gain reproductive success through extrapair fertilizations in the stitchbird. *Animal Behaviour*, **58**, 321–328.

Frankham R (1996) Do island populations have less genetic variation than mainland populations? *Heredity*, **78**, 311–327.

Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous Red-winged Blackbirds revealed by DNA markers. *Science*, **250**, 1394–1397.

Gowaty PA, Bridges WC (1991) Nestbox availability affects extrapair fertilizations and conspecific nest parasitism in Eastern Bluebirds *Sialia sialis*. *Animal Behaviour*, **41**, 661–675.

Griffith SC, Stewart IRK, Dawson DA, Owens IPF, Burke T (1999) Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? *Biological Journal of the Linnean Society*, **68**, 303–316.

Griffith SC (2000) High fidelity on islands: a comparative study of extrapair paternity in passerine birds. *Behavioral Ecology*, **11**, 265–273.

Kempenaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? *Behavioral Ecology*, **10**, 304–311.

Lifjeld JT, Dunn PO, Robertson RJ, Boag PT (1993) Extra-pair paternity in monogamous tree swallows. *Animal Behaviour*, **45**, 213–229.

Lifjeld JT, Robertson RJ (1992) Female control of extrapair fertilization in tree swallows. *Behavioural Ecology and Sociobiology*, **31**, 89–96.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Meagher TR (1986) Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. *American Naturalist*, **128**, 199–215.

Mitchell JS, Robertson RJ (1996) Extra nest site occupancy by tree swallows: do floaters avoid nest sites near settled pairs? *Wilson Bulletin*, **108**, 797–802.

Møller AP (1985) Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the Swallow *Hirundo rustica*. *Behavioural Ecology and Sociobiology*, **17**, 401–408.

Møller AP (1991) Density-dependent extrapair copulations in the Swallow *Hirundo rustica*. *Ethology*, **87**, 316–329.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.

Owens IPF, Hartley IR (1998) Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proceedings of the Royal Society of London Series B*, **265**, 397–407.

Paynter RA (1954) Interrelationships between clutch-size, brood-size, prefledging survival, and weight in Kent Island tree swallows. *Bird Banding*, **25**, 35–148.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249–252.

Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology and Evolution*, **13**, 52–58.

Primmer CR, Møller AP, Ellegren H (1995) Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. *Molecular Ecology*, **4**, 493–498.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Robertson RJ, Stutchbury BJ, Cohen RR (1992) The tree swallow (*Tachycineta bicolor*). In: *The Birds of North America* (eds Poole A, Stettenheim P, Gill F) vol. 11, pp. 1–28. Academy of Natural Sciences, Philadelphia and American Ornithologists' Union, Washington, DC.

Stutchbury BJ, Robertson RJ (1985) Floating populations of female Tree Swallows. *Auk*, **102**, 651–654.

Westneat DF, Sherman PW, Morton ML (1990) The ecology and

evolution of extrapair copulations in birds. *Current Ornithology*, **7**, 331–369.

Westneat DF, Sherman PW (1997) Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioural Ecology and Sociobiology*, **41**, 205–215.

Wheelwright NT, Leary J, Fitzgerald C (1991) The costs of reproduction in tree swallows (*Tachycineta bicolor*). *Canadian Journal of Zoology*, **69**, 2540–2547.

Wilkinson L (1992) *Systat for Windows, Version 5*. Systat Inc., Evanston, IL.

Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*, **129**, 157.