

# Multiple paternity and offspring quality in tree swallows

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**Abstract** There is mounting evidence in a variety of taxa that females increase offspring quality by mating with multiple males, often resulting in multiple paternity. In birds, however, few studies have explicitly examined the benefits of mating with several different males; instead, the focus has been on whether or not extra-pair mating occurs, and its adaptive significance remains controversial. We examined the hypothesis that offspring quality, particularly immune response (phytohaemagglutinin assay) and growth, increases with the number of sires in broods of socially monogamous tree swallows (*Tachycineta bicolor*). We found one of the highest known levels of multiple paternity in birds (84% of nests with two or more extra-pair young had at least two extra-pair sires). Among nests with extra-pair young, the number and diversity of sires continued to increase linearly with the number of extra-pair young, so there was no evidence that some males monopolized paternity at high levels of extra-pair fertilization. Indeed, the number of sires was actually greater than expected in large broods, suggesting that some females might be seeking more mates. We found no effect of the number of sires on nestling immune response or growth. In mixed paternity broods, the immune response of extra-pair young did not differ from that of their within-pair half-siblings.

However, among all broods, nestlings had a stronger immune response in nests with at least one extra-pair nestling than in nests with all within-pair nestlings. These results are not consistent with a good genes benefit of extra-pair mating, but they do suggest that there are environmental effects associated with extra-pair mating that increase nestling immune response. These environmental effects could produce indirect genetic effects on sexual selection if they are heritable. The extraordinarily high number of sires in this species highlights a relatively unexplored source of sexual selection in birds.

**Keywords** Extra-pair paternity · Genetic benefits · Immune response · Genetic polyandry

## Introduction

In many species, females have multiple sires within a brood, but the adaptive significance of this multiple paternity (genetic polyandry) remains controversial (Jennions and Petrie 2000; Simmons 2005). Given that multiple mating is likely to benefit males more than females, we might expect sexual conflict over the number of sires. If males often harass females while seeking copulations, then mating with several males may reduce the costs of harassment or injury to females. Mating with different males could also provide some direct benefits to females such as ensuring the entire clutch is fertilized, protection from predators, or, in some invertebrates, nuptial gifts. Several genetic benefit hypotheses have also been proposed to explain multiple mating in cases where females receive only sperm from males, and there appear to be no direct benefits. These hypotheses propose that mating with different males increases the probability of fertilization by superior sperm in terms of

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additive genetic effects (trading up, post-copulatory choice, good sperm hypotheses; Yasui 1998; Jennions and Petrie 2000; Simmons 2005) or non-additive effects, such as increasing mean offspring genetic diversity (Baer and Schmid-Hempel 1999; Hoffman et al. 2007) or compatibility between parental genotypes (inbreeding avoidance and compatibility hypotheses; Zeh and Zeh 1996). These genetic benefit hypotheses typically predict that females producing broods with more sires will have greater fitness because their offspring are of higher quality. This prediction is based on the assumption that females allocate more fertilizations to particular males, which may occur through active choice or cryptic mechanisms of sperm selection. If females mate with numerous males without any discrimination or biases in fertilization (bet-hedging), then there is no reason to predict that offspring of multiple mating females will be of higher quality (Yasui 1998). Some experimental studies have found evidence that multiple paternity improves offspring quality (reviewed by Simmons 2005; Fisher et al. 2006), but evidence that females actively choose and benefit from multiple mating in the wild is still controversial (Arnqvist and Kirkpatrick 2005; Griffith 2007; Mäkinen et al. 2007; DiBattista et al. 2008).

Field studies suggest that multiple paternity leads to greater offspring viability in arthropods (reviewed in Simmons 2005), reptiles (Madsen et al. 1992; Olsson et al. 1994), fish (Garant et al. 2005), and mammals (Hoogland 1998; Fisher et al. 2006). Despite the large number of studies of extra-pair paternity in birds, to date, only one study has examined the relationship between the number of sires per brood and offspring fitness. Schmoll et al. (2007) found no association between the number of sires and offspring recruitment in coal tits (*Parus ater*); however, females rarely maximized the diversity of sires in their broods. Although most (66%) females had extra-pair young in their nest and they laid up to nine eggs, there was a maximum of only three sires in a brood (Schmoll et al. 2007).

In this study, we examined the hypothesis that extra-pair paternity and the number of sires, in particular, increases offspring quality (immunocompetence, heterozygosity, and growth) in tree swallows (*Tachycineta bicolor*). Multiple paternity is often used to refer to the presence of extra-pair mating in birds, but there are often only two sires (the within- and extra-pair male) in broods with extra-pair young. Here, we use the term multiple paternity to focus on the total number of sires. Tree swallows are particularly well suited for this study because they have one of the highest reported levels of both extra-pair mating (83% of broods contain extra-pair young) and multiple paternity in birds. There are at least three different sires in 72% of tree swallow broods with multiple extra-pair young, and up to six different males may sire a brood of six young (Whittingham et al. 2006). These high levels of multiple

paternity suggest that mating with several different males may have important fitness consequences for females.

One possible fitness benefit of a large number of sires is that it increases the probability that at least one nestling will have superior qualities. If females have few phenotypic cues to the genetic quality of males, then the best tactic for females may be to mate with a number of different males to increase the probability that at least one of them supplies a superior genotype for her offspring (Yasui 1998, 2001). This tactic would most likely require some type of sperm selection after copulation (Yasui 1998, 2001), perhaps greater fertilization success of more competitive sperm or gametes from more compatible maternal and paternal genotypes (Firman and Simmons 2008). In this case, it may be the maximum quality of any nestling that has the greatest effect on female fitness, particularly if few young in a brood are likely to recruit. To test this hypothesis, we examined the maximum immunocompetence and growth rate of any nestling in the brood in relation to the number of sires in the brood.

We also examined how the number and distribution of sires within a brood increases with the number of extra-pair young. If paternity is based on a fair raffle process, then we would expect more sires in larger broods, simply because there are more opportunities for any male to fertilize eggs (Parker 1990). There should also be little skew in the distribution of male paternity (i.e., high evenness) within a brood if each male has an equivalent chance of fertilization. Alternatively, if males monopolize paternity in a brood, then the relationship between number of sires and number of extra-pair young should be non-linear, possibly reaching a threshold number of sires at intermediate or high levels of extra-pair paternity or even decreasing at high levels.

## Methods

### Study site and species

In 2000, we studied tree swallows breeding in 88 nest boxes at the University of Wisconsin-Milwaukee Field Station near Saukville, Wisconsin, USA (43°23' N, 88°01' W). In our population, tree swallows are predominantly socially monogamous and single brooded. Females build a grass cup nest inside the nest box, lay three to seven eggs (one egg per day) in early May and begin incubation (14–15 days) with the penultimate egg. Young remain in the nest for 18–22 days after hatching (Robertson et al. 1992).

All adults were caught inside nest boxes and measured (wing cord and tarsus length), weighed, and marked with a U.S. Fish and Wildlife Service band on the right leg and a single colored plastic band on the left leg. In addition, a small (50 µl) blood sample was collected by brachial

venipuncture for molecular analyses (see below). Sex of adults was determined by the presence of a brood patch or cloacal protuberance, and females were classified as second-year (SY) or after second-year (ASY) on the basis of plumage coloration (Hussell 1983).

Nest boxes were inspected daily to determine the start of egg laying and clutch size. We checked nests every 1.5–2 h on the expected day of hatching to determine the time of hatching of each egg. Each egg was weighed on an electronic balance ( $\pm 0.01$  g) on the morning it was laid. All nestlings were marked at hatching by clipping a toenail, and we could identify individuals throughout the nestling period. Each nestling was weighed at hatch and on days 2, 4, 6, 10, and 12 post-hatch using an electronic balance ( $\pm 0.01$  g). Growth rate for each nestling was calculated as the mass increase divided by the time span (in hours) between measurements. On day 12, nestlings reach the asymptote of their adult size (Zach 1982). At this age, we weighed and measured nestlings and collected a small (50  $\mu$ l) blood sample for molecular analyses (see below). Blood samples were stored in lysis buffer at 4°C. All unhatched eggs and dead nestlings were collected, and tissues were frozen at  $-20^\circ\text{C}$ .

#### Immunoassay

In birds, the strength of the cellular immune system is often measured by the size of the swelling in response to a subcutaneous injection of a non-pathogenic antigen, phytohaemagglutinin (PHA) that causes a local swelling. This PHA response is correlated positively with nestling survival (Christe et al. 2001) and disease resistance (Lochmiller et al. 1993). We tested the immunocompetence of nestlings with a subcutaneous injection of 0.2 mg PHA dissolved in 40  $\mu$ l of saline into the right wing. A control of 40  $\mu$ l of saline was injected into the same location on the left wing. Wing thickness was measured to the nearest 0.01 mm before injection on day 10 post-hatch and 24 ( $\pm 1$ ) h later using a micrometer (Teclock, model SM-112) with a rounded tip (Liffield et al. 2002). For simplicity, we used the increase in wing thickness at the PHA injection site (right wing) as our measure of immune response because there was no detectable change in wing thickness as the result of the control (saline) injection, and there was a strong correlation between estimates of the swelling using just the right wing and estimates based on the difference in swelling between the right and left wings (Whittingham et al. 2007). The one-wing technique is well documented as an appropriate technique for characterizing PHA-induced wing web swelling in birds (Smits et al. 1999; Martin et al. 2006). All measurements of immune response were performed by JTL. Air temperature can influence the PHA response of adult tree swallows (Liffield et al. 2002),

so our analyses controlled for mean daily air temperature, which we obtained from an automated weather station located half-way between our two nest-box grids.

#### Genetic analyses

Paternity was determined from analysis of allelic variation at seven microsatellite loci (HrU6, Ltr6, Tbi-104, Ibi5-29, Ase29, Ase55, and Pat43). Details of the PCR conditions and thermal cycling protocols for HrU6, Ltr6, Tbi-104, and Ibi5-29 are provided in Whittingham et al. (2006). PCR reactions for Ase29, Ase55 (Richardson et al. 2000), and Pat43 (Otter et al. 1998) consisted of 50 ng of template DNA, 0.5  $\mu$ M of each primer (the forward primer was fluorescently labeled; Applied Biosystems), 0.2 units of DNA polymerase (DyNAzyme, Finnzymes) in the manufacturer's buffer (final concentration of 10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.1% Triton X-100), and 0.2 mM dNTP mix. We used a touchdown thermal profile for both Ase29 and Ase55 as follows: initial denaturation at 94°C for 3 min, followed by 15–17 (respectively) cycles of 94°C for 30 s, 55–45°C for 45 s, 72°C for 45 s, and a final step at 72°C for 5 min. The annealing temperature was reduced by 1°C every 2 cycles. For Pat43, we used an initial denaturation of 94°C for 3 min, and 32 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 40 s, and a final step at 72°C for 5 min. Amplified PCR products were run on an ABI 373 automated sequencer and analyzed with Genescan Analysis and Genotyper software (PE Biosystems).

The frequency of each allele was calculated for each locus from the genotypes of at least 120 adults (see Whittingham et al. 2006 and Stapleton et al. 2007 for more details). These frequencies were used to calculate the total exclusion probability of all loci combined ( $P_{\text{ct}}$ ), which was 0.993 with one parent known (Jamieson 1994). There was no evidence of null alleles in the families we studied (maternal alleles appeared to amplify in all offspring), and the estimated frequency of null alleles ( $r$ ; Brookfield 1996) was low at all loci (all  $r < 0.025$ ). Note that in this study we excluded loci that have previously shown evidence of null alleles or were not in Hardy–Weinberg equilibrium (HrU7, Ppi2; Whittingham et al. 2006). Heterozygosity of individuals was calculated as the proportion of heterozygous loci (at all seven loci) divided by the observed mean heterozygosity of all loci (i.e., standardized heterozygosity; Coltman et al. 1999).

All young matched their putative mother at all seven loci. Nestlings were considered within-pair young if they matched the putative father at all seven loci and extra-pair young if they mismatched the putative father at two or more loci. For offspring that mismatched their putative father at just one locus ( $N=8$ ), we calculated the probability of chance inclusion, which is the probability that a random

male would match the nestling (Jeffreys et al. 1992), at the six matching loci. Three young had a low probability of chance inclusion ( $<0.05$ ; range=0.033–0.049) and were considered within-pair young. These single mismatches occurred at different loci and were considered mutations or genotyping errors. The remaining five young had a high probability of chance inclusion ( $>0.05$ ; range 0.40–0.679) and were considered extra-pair young (Johnsen et al. 2000). In these five young, the size of the mismatched allele was identical to the paternal allele of another extra-pair nestling in the same brood.

To assign extra-pair sires, the paternal alleles of the extra-pair young were compared to the alleles of all males sampled in the population ( $n=60$ ) at all seven loci. Extra-pair sires were assigned only when they matched an extra-pair young at all seven loci. Of 34 broods with extra-pair young, we could assign all or all but one extra-pair young to an extra-pair sire for 26 broods and thus determine the total number of sires contributing to each brood. The remaining eight broods had at least two extra-pair young with unknown sires. In these cases, we estimated the number of unknown extra-pair sires by estimating whether the nestlings in the brood were full- or half-siblings using the computer program Kinship 1.2 (Goodnight and Queller 1999). The relatedness estimates were based on known maternal and nestling genotypes and population allele frequencies generated from 120 unrelated adult birds at the seven microsatellite loci above. For each pairwise comparison of nestlings, Kinship produces a likelihood ratio for two hypotheses. In this case, the first (primary) hypothesis was that the two extra-pair young were full sibs (shared the same extra-pair father), and the second (null) hypothesis was that they were half-sibs (shared the same mother, but different extra-pair sires). We then used the simulation routine in Kinship to perform a significance test of the likelihood ratio for each pair of nestlings (a high value indicates stronger support for the primary hypothesis that extra-pair young were full sibs). We considered unassigned extra-pair young to be full sibs if their observed likelihood ratio exceeded 95% of the simulated null hypothesis pairs (Whittingham et al. 2006). Thus, we could determine the total number of sires in each brood by combining the number of assigned resident sires and the number of unknown sires.

The genetic diversity hypothesis assumes that multiple paternity produces significant increases in within-brood genetic diversity (Boag and Ratcliffe 2000). To test this assumption with our genetic markers, we calculated the mean number of alleles per locus and allelic richness (Petit et al. 1998) using the pooled sample of all the nestlings in each brood. Allelic richness provides a measure of allelic variation that takes into account unequal numbers of nestlings in each brood using the technique of rarefaction. Allelic richness was calculated using the program FSTAT (Goudet 1995).

We determined the sex of tree swallow young using primers (P2 and P8) that amplify an intron of the CHD1 gene on the avian sex chromosomes (Griffiths et al. 1998). The details of our protocol for tree swallows are described in Whittingham and Dunn (2000).

#### Statistical analyses

In this study, each male and female pair had only one nest and, thus, nests were independent. Sire diversity within each brood was estimated using the number of sires, as well as the Shannon–Wiener  $D$  index, which was recently used by Schmoll et al. (2007) to estimate the degree of genetic polyandry in coal tits. The  $D$  index is based on information theory and in this context provides an index of the number of sires that accounts for the number of young sampled.  $D$  was calculated for each brood as  $-1(p_i \times \ln p_i)$  summed for all  $s$  sires in a brood, where  $p_i$  is the proportion of young sired by the  $i$ th sire in a brood. We also estimated sire evenness ( $E$ ), which reflects the degree to which paternity has been divided among a given number of sires. Evenness was calculated as  $D$  divided by  $\ln(s)$ . Note that a brood of six young will have the same evenness (1.0) if there are six different sires or there are two sires that each produce three young. However, sire diversity is much greater in the first (1.79) than the second (0.69) case.

We used a randomization procedure to test whether the total number of sires in a brood (both within- and extra-pair) was similar to a fair raffle process (Parker 1990) or whether females were mating with more males than expected under random fertilization. A positive relationship between the number of sires and brood size is expected as a sampling artifact, simply because there are more opportunities for any male to fertilize eggs in a larger brood. Thus, we calculated a bootstrap confidence interval (CI) and compared it to the observed mean for each brood size (3 to 6) in our sample. For each of the bootstrap replicates, we generated 41 simulated broods, which was the number of nests in our sample. The simulation took each of the 41 nests and disassociated the number of young at the nest from the number of sires (see also Neff et al. 2008). It assigned a number of sires to each nest from a randomly chosen nest (with replacement). The number of sires at this randomly chosen nest was used if there were fewer young in the random nest than in the focal nest. However, if there were more young in the random nest than in the focal nest, we randomly chose sires from the randomly chosen nest until all of the young in the focal nest had been assigned sires. The same procedure was used when nests had equal numbers of young. The randomization procedure was repeated for all 41 broods in our sample, and a mean number of sires was calculated for each brood size. This procedure was repeated 1,000 times, and a bootstrap 95%

CI for the number of sires was obtained for each brood size from the 0.025 and 0.975 quantiles of the 1,000 replicates (Whitlock and Schluter 2009). For each brood size, we compared the observed mean number of sires with the bootstrap 95% CI to determine whether the number of sires was similar to a fair raffle process.

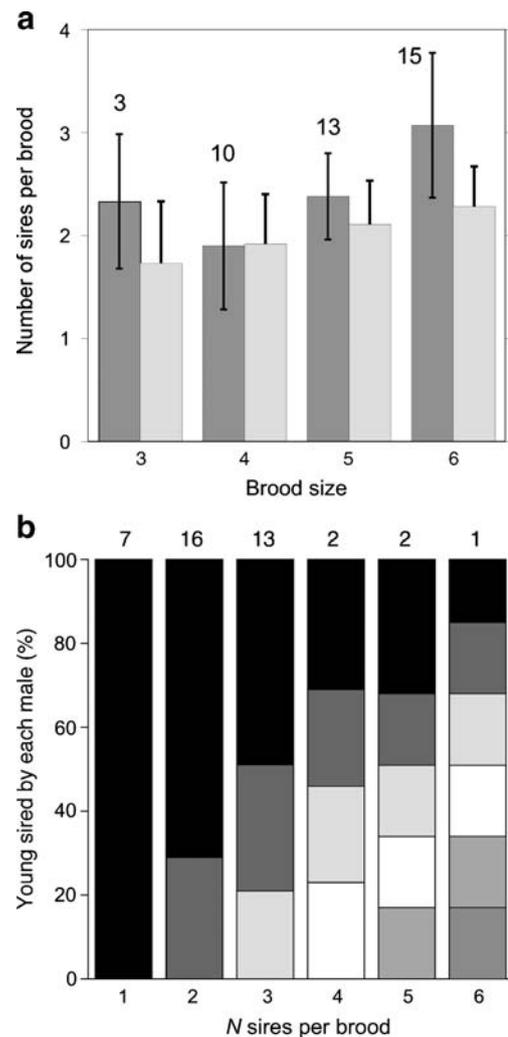
To test the hypothesis that multiple paternity increases maximum nestling quality, we examined the maximum PHA response and growth rate of any nestling in the brood in relation to the number of sires (one, two, and greater or equal to 3) in the brood. The analysis included the number of young in each brood to control for the likely increase in maximum values in broods with more young. Statistical analyses were performed with JMP (SAS Institute 2003). All tests were two-tailed, and means are reported with their standard errors, unless indicated otherwise.

## Results

As found in previous studies of this species, tree swallows had high levels of extra-pair mating: 83% of broods contained extra-pair young (34 of 41 broods) and 49% (101 of 206) of young were sired by extra-pair males. Seven broods contained only within-pair young, seven broods contained only extra-pair young (all with multiple extra-pair sires), and 27 broods had mixed paternity (within and extra-pair young). Overall, 17% (seven of 41) of broods were sired entirely by the within-pair male, 31% (13 of 41) of broods had one extra-pair sire, and 51% (21 of 41) of broods had at least two different extra-pair sires. The total number of sires (including the within-pair male) averaged  $2.5 \pm 0.18$  males per brood (broods averaged  $5.0 \pm 0.15$  young in this sample). The number of extra-pair sires averaged  $1.7 \pm 0.20$  (range 0–5;  $N=41$ ) males per brood, including all broods, and  $2.1 \pm 0.20$  (range 1–5;  $n=34$ ) males per brood when we only included broods with extra-pair young.

The total number of sires increased with brood size, as expected ( $r^2=0.12$ ,  $F_{1,39}=5.5$ ,  $P=0.02$ ), and in most brood sizes, the observed number of sires was not different from random (i.e., they overlapped with the 95% bootstrap CI from our simulations; Fig. 1a). However, in broods of six young, females had more sires than would be expected by chance ( $P<0.05$  based on a bootstrap 95% CI; Fig. 1a).

We found at least two extra-pair sires in 84% (21 of 25) of nests with two or more extra-pair young. On average, in broods with two sires, one male sired most of the young (71%), typically the within-pair male (ten of 16 broods; Fig. 1b). In broods with three sires, the most successful male was usually extra-pair (eight of 13 broods), siring an average of 49% of the young. Broods with four to six sires had relatively even proportions of young sired by each

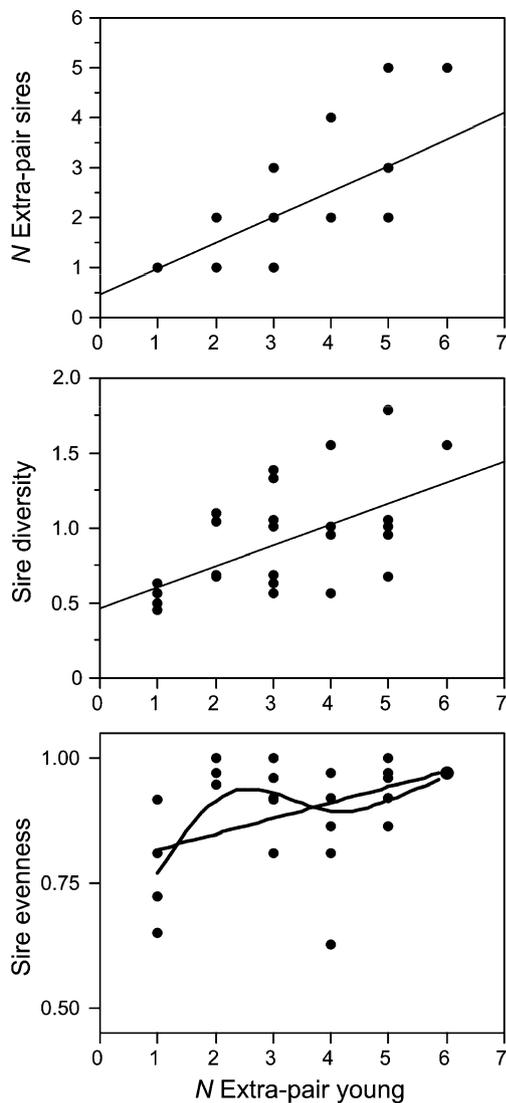


**Fig. 1** **a** Mean ( $\pm 95\%$ CI) number of sires per brood in observed (dark bars) and random (light bars) nests. Random distributions were simulated with 1,000 sets of similar sized broods (see “Methods”). Numbers of observed broods are indicated above the bars. **b** Mean percentage of young sired by different males within broods of tree swallows. In each brood category, the first male sired the largest percentage of young (black bars), the second male sired the next largest percentage of young (dark gray), the third male sired the third largest percentage of young (stippling), and so forth. Number of broods in each category is indicated at the top of each bar

male, as might be expected when the number of sires approaches the number of young.

The total number of sires in a brood was highly correlated with the sire diversity index ( $r=0.97$ ,  $n=41$ ,  $P<0.001$ ), but the correlation was weaker with sire evenness ( $r=0.45$ ,  $n=34$ ,  $P=0.008$ ). Sire diversity and evenness averaged  $0.73 \pm 0.073$  ( $n=41$ ) and  $0.88 \pm 0.020$  ( $n=34$ ), respectively. Note that sample sizes of broods differ because evenness is undefined for broods with a single sire. For comparison, a typical brood of five nestlings with one extra-pair young would have a sire diversity and evenness of 0.32 and 0.46, respectively. However, if all five nestlings have different

sires, the values increase to 1.61 and 1.0, respectively. Of the broods with extra-pair young, 62% (21 of 34) had evenness values  $>0.9$ , and 53% (18 of 34) had sire diversity values  $>0.9$ . Among nests with extra-pair young, the number and diversity of sires continued to increase linearly with the number of extra-pair young and did not reach a threshold or drop off at high levels of extra-pair paternity (Fig. 2). Thus, more sires were present as the number of



**Fig. 2** Number of extra-pair sires ( $r^2=0.56$ ,  $n=34$ ,  $F_{1,32}=40.3$ ,  $P<0.001$ ), sire diversity ( $r^2=0.40$ ,  $n=34$ ,  $F_{1,32}=21.1$ ,  $P<0.001$ ), and sire evenness ( $r^2=0.18$ ,  $n=34$ ,  $F_{1,32}=7.0$ ,  $P=0.013$ ) increased with the number of extra-pair young in a brood. Seven nests without extra-pair young were excluded from analysis. Straight lines are from linear regressions. Quadratic regressions (second-order polynomial) did not explain significantly more variation than linear regressions for the number of extra-pair sires ( $r^2=0.57$ ) or sire diversity ( $r^2=0.41$ ). However, there were significant increases in the variation in sire evenness explained by second- (adjusted  $r^2=0.23$ ), third- (adjusted  $r^2=0.41$ ), and fourth-order ( $r^2=0.47$ ) polynomials. A spline is shown with similar  $r^2$  (0.47;  $\lambda=2$ )

extra-pair young increased, and, as a consequence, sire evenness remained at high levels among broods with at least two extra-pair young (Fig. 2). Thus, there was no evidence that a few males monopolized paternity at high levels of extra-pair mating.

Next, we examined the relationship between multiple paternity and nestling quality in terms of immune response as assayed by the PHA test. The PHA response of individual nestlings was analyzed with a mixed model (nest was random effect) containing several fixed effects (Table 1), including total number of sires in the brood, sire diversity ( $D$ ), the type of brood (all within-pair young, mixed paternity or all extra-pair young), paternity of the nestling (within-pair or extra-pair), nestling sex, brood size, body mass (day10), air temperature (day10), laying date (to control for time of season), and length of the ninth primary feather (day12, to control for body size). In the initial model with all predictors, PHA response was lower in birds with longer ninth primaries ( $P<0.01$ ), and PHA response was greater though not significantly in birds that weighed more ( $P=0.06$ ; Table 1). These patterns were also found in the reduced model (all  $P<0.07$ ) along with an effect of brood type ( $P=0.02$ , Table 1). On average, the PHA response of nestlings was greater in broods with all extra-pair young ( $1.1\pm 0.04$  mm, least squares mean $\pm$ SE) than in broods with all within-pair young ( $0.88\pm 0.04$  mm; Tukey–Kramer test; Fig. 3). Broods with any number of extra-pair young (all extra-pair young and mixed paternity broods) also had a greater though not significant PHA response than broods with all within-pair young ( $F_{1,152}=6.8$ ,  $P=0.07$ ). The effect of paternity on PHA response did not appear to be the result of differences in initial maternal investment as egg mass (at laying) did not vary with brood type ( $F_{2, 157}=0.98$ ,  $P=0.38$ ) in a mixed model that also included laying date ( $F_{1, 157}=0.30$ ,  $P=0.58$ ) and sex of the nestling ( $F_{1, 157}=0.32$ ,  $P=0.57$ ).

The maximum PHA response of any nestling in the brood was also affected primarily by whether or not there were any extra-pair young in the brood. Maximum PHA response of any nestling was related positively to the number of sires (coded as one, two, or greater or equal to three sires;  $F_{2, 36}=4.0$ ,  $P=0.027$ ) in a model that also included air temperature ( $F_{1, 36}=9.4$ ,  $P=0.004$ ) on day 10 and brood size ( $F_{1, 36}=0.1$ ,  $P=0.81$ ). However, this effect of the number of sires on maximum PHA response was due to broods with all within-pair young as the effect of number of sires became non-significant when those broods were excluded from the analysis ( $F_{1, 30}=1.9$ ,  $P=0.18$ ). Overall, these results suggest that the effect of extra-pair paternity (some/none) on mean PHA response (Fig. 3a) was driven by the higher maximum PHA values in broods containing extra-pair young.

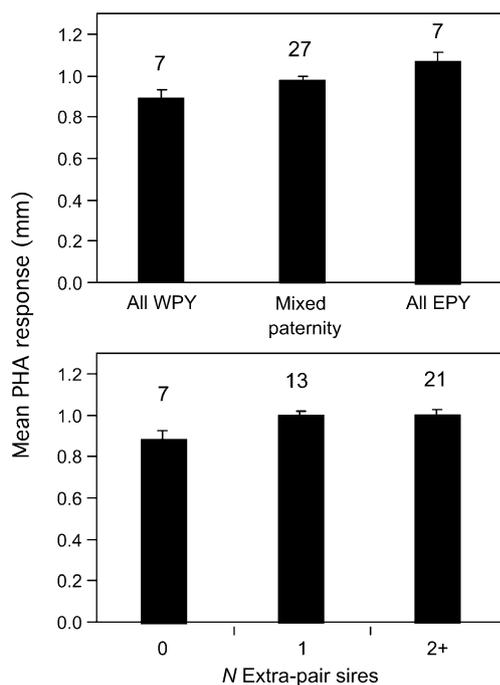
In an analysis of half-sibs (using mixed paternity broods), which controls for maternal genotype and nest environment, PHA response was not related to paternity

**Table 1** Mixed model analysis of PHA response of individual nestlings on days 10–11

Variable	Full model		Reduced model		Bivariate models		
	Slope	<i>P</i>	Slope	<i>P</i>	Slope	<i>P</i>	$r^2$
Total sires ( <i>n</i> )	<-0.01	0.61			0.01	0.57	0.14
Sire diversity ( <i>D</i> )	0.03	0.91			0.04	0.31	0.15
Sire evenness ( <i>E</i> )	–	–			-0.09	0.60	0.11
Paternity (WP/EP)	0.01	0.58			0.03	0.10	0.14
Brood type (all WPY, mixed, all EPY)	–	0.14	–	0.02	–	0.02	0.15
Body mass (d 10)	0.02	0.06	0.02	0.07	<0.01	0.57	0.14
Brood size (d 11)	0.01	0.56			-0.01	0.50	0.14
Air temperature (d 11)	<0.01	0.56			<0.01	0.44	0.14
Sex	0.02	0.36			<0.01	0.81	0.11
Laying date	<0.01	0.19			<0.01	0.41	0.14
Ninth primary length (d 12)	-0.01	<0.01	-0.01	<0.01	<-0.01	0.03	0.15
$R^2$	0.18		0.17				
<i>n</i>	188		195				

The full model contained all of the variables on the left (except sire evenness), and the reduced model contained all of the variables with  $P < 0.15$  after stepwise elimination from the full model. All analyses were mixed models with nest as a random effect. Bivariate models were conducted separately with each variable on the left and also included nest as a random effect. 'd' refers to nestling age in days. Sire evenness was not included in the full model because it was only calculated for nests with more than one sire

(within-pair or extra-pair;  $F_{1, 99} = 0.24$ ,  $P = 0.62$ ) or the number of sires ( $F_{1, 99} = 1.0$ ,  $P = 0.31$ ) in a model that also included nestling mass ( $F_{1, 99} = 0.32$ ,  $P = 0.57$ ) and air temperature ( $F_{1, 99} = 2.0$ ,  $P = 0.16$ ) on day 10.



**Fig. 3** Mean PHA response (mm) in relation to type of paternity in a brood ( $F_{2,38} = 4.2$ ,  $P = 0.023$ ) and number of extra-pair sires in the brood ( $F_{2,37} = 3.09$ ,  $P = 0.058$ , in a model with brood size). Bars indicate the mean  $\pm$  1 SE. Number of broods in each category is indicated at the top of each bar

Growth rate of nestlings from days 6 to 12 (when it is increasing linearly) was not related to the number or diversity of sires in the brood. In a mixed model analysis (Table 2), growth rate was related to brood size, hatching order, mass at hatching, sex, and length of the ninth primary, but not related to the number of sires, paternity, brood type, or hatching date. There was also no effect of the number of sires in an analysis of half-sibs using mixed paternity broods ( $F_{1, 99} = 2.5$ ,  $P = 0.12$ ). The maximum growth rate of any nestling in a brood was also not related to the number of sires (coded as one, two, or greater or equal to three sires;  $F_{2, 35} = 0.9$ ,  $P = 0.40$ ) in a model that included the number of young in the brood ( $F_{1, 35} = 0.1$ ,  $P = 0.84$ ).

Lastly, we examined levels of genetic variation in relation to number of sires and paternity. Within broods, the mean number of alleles per locus increased linearly with the number ( $r^2 = 0.30$ ,  $F_{1,38} = 5.9$ ,  $P = 0.02$ ) and diversity ( $r^2 = 0.33$ ,  $F_{1,38} = 8.3$ ,  $P = 0.007$ ) of sires after controlling for brood size. Allelic richness increased with the diversity ( $r^2 = 0.11$ ,  $F_{1,39} = 5.0$ ,  $P = 0.03$ ) of sires, but not with the number ( $r^2 = 0.08$ ,  $F_{1,39} = 3.2$ ,  $P = 0.08$ ) of sires. There was no evidence that these estimates of genetic variation reached a threshold at high levels of extra-pair mating (a second order polynomial did not explain more variation). In a comparison of half-sibs, individual heterozygosity of nestlings was not related to either paternity (within-pair or extra-pair;  $F_{1,106} < 0.01$ ,  $P = 0.96$ ) or the number of sires in the brood ( $F_{1,106} = 0.55$ ,  $P = 0.46$ ; mixed model with nest as the random effect).

**Table 2** Mixed model analysis of individual nestling growth (g/day) during days6–12

Variable	Full model		Reduced model		Bivariate models		
	Slope	<i>P</i>	Slope	<i>P</i>	Slope	<i>P</i>	$r^2$
Total sires ( <i>n</i> )	0.02	0.91			0.05	0.18	0.66
Sire diversity ( <i>D</i> )	0.12	0.83			0.13	0.21	0.65
Sire evenness ( <i>E</i> )	–	–			0.33	0.46	0.64
Paternity (WP/EP)	–	0.33			–	0.46	0.65
Brood type (all WPY, mixed, all EPY)	–	0.89			–	0.43	0.66
Sex	–	<0.01	–	<0.01	–	<0.01	0.67
Brood size	–0.12	0.03			–0.05	0.25	0.66
Hatch date	–0.01	0.11			–0.01	0.16	0.66
Hatch mass	–0.40	<0.01	–0.35	0.01	–0.34	0.04	0.67
Hatch order	0.05	<0.01	0.05	<0.01	0.06	<0.01	0.74
Ninth primary length (d12)	<–0.01	0.046	<–0.01	0.048	–0.02	<0.01	0.71
$R^2$	0.77		0.77				
<i>n</i>	185		187				

The full model contained all of the variables on the left (except sire evenness), and the reduced model contained all of the variables with  $P < 0.15$  after stepwise elimination from the full model. All analyses were mixed models with data from individual nestlings and nest as a random effect. Bivariate models were conducted separately with each variable on the left and included nest as a random effect. ‘d’ refers to nestling age in days. Sire evenness was not included in the full model because it was only calculated for nests with more than one sire

## Discussion

Multiple paternity has been studied in many taxa (reviewed by Simmons 2005; Fisher et al. 2006), but it has rarely been examined in birds (Schmoll et al. 2007). Few studies of extra-pair mating in birds present data on the number of sires, but the available evidence suggests that there is often only one extra-pair sire. In contrast, tree swallows have both high levels of extra-pair mating (83% of broods) and a large number of sires (there were at least two extra-pair sires in 84% of broods with multiple extra-pair young). Several indirect genetic benefit hypotheses predict that multiple paternity will lead to greater offspring quality, but we did not find any support for this prediction when we compared half-sibs sired by extra- and within-pair males. This lack of difference would be predicted if females mated indiscriminately with extra-pair males, perhaps if it increases the probability that at least one of them will provide a superior genotype for her extra-pair offspring (Yasui 1998; 2001). In this scenario, females might follow a genetic bet-hedging strategy, but theory indicates within-generation bet-hedging is unlikely to evolve because under random mating, a polyandrous female is just as likely to mate with a low quality male as a high-quality male (Yasui 2001; Hopper et al. 2003). Indeed, we found little evidence that the number of sires was related to the average or maximum quality of offspring; the differences we found in PHA response were primarily associated with whether or not there were any extra-pair young in the brood. On the

other hand, recent studies of tree swallows suggest that females do not mate randomly, and some genetic benefits of extra-pair mating exist, but they might be dependent on environmental conditions (O’Brien and Dawson 2007; Stapleton et al. 2007). Below, we examine these and other hypotheses and their implications for multiple paternity.

One of the simplest explanations for multiple paternity is that it is a consequence of selection on males for mating with many females. In this case, multiple mating may provide little or no benefit to females, and it may simply be a consequence of females accepting matings because the costs of resistance (avoiding harassment) are greater than the cost of mating itself. This type of mating has been called “convenience polyandry”, and it has been suggested in some invertebrates (Maklakov and Lubin 2004), turtles (Lee and Hays 2004), and sharks (DiBattista et al. 2008) where researchers found no evidence for indirect genetic benefits. Harassment can be persistent and dangerous in some birds such as waterfowl (McKinney and Evarts 1998; Dunn et al. 1999); however, our observations do not suggest that harassment leads to convenience polyandry in tree swallows.

Female tree swallows copulate frequently on top of their nest box prior to laying, and behavioral observations indicate that females do not mate indiscriminately with extra-pair males (Venier et al. 1993). Experimental evidence also suggests that females exert significant control over which males fertilize their clutch (Lifjeld and Robertson 1992). Furthermore, the number of different sires is repeatable

between broods of the same female, but the particular male siring young is not repeatable (Whittingham et al. 2006). If male coercion was common, then we might expect to see the same males siring young in successive broods (i.e., paternity should be repeatable), but this did not occur (Whittingham et al. 2006). The greater than expected number of sires in large broods (six young; Fig. 1a) and the positive relationships between level of extra-pair paternity and both diversity of sires and evenness of paternity (Fig. 2) also suggest that males were not monopolizing fertilizations. Indeed, females with larger broods, which are also older individuals (Robertson and Rendell 2001), might be better able to gain more sires in their broods. Female guppies (*Poecilia reticulata*) with larger broods also had more sires than expected, but it was not clear if this was due to female preferences or forced copulations by males (Neff et al. 2008), which are unknown in tree swallows. If male coercion is unlikely in tree swallows, then why do females mate with so many males when compared with other socially monogamous species, even ones that inhabit the same study area and also use nest boxes, such as eastern bluebirds (*Sialia sialis*; Meek et al. 1994) and house wrens (*Troglodytes aedon*; Poirier et al. 2004)?

One possible difference between tree swallows and other species is their high rate of within-pair copulation (Venier and Robertson 1991), which could lead to sperm depletion and temporary infertility. Fertility insurance has been suggested as a potential direct benefit of genetic polyandry in several species. In a recent study of polygynous red bishops (*Euplectes orix*), Edler and Friedl (2008) found that within-pair young had a greater PHA response than their extra-pair half-siblings. They suggested that this pattern arose because high-quality males with several mates are more likely to have temporary infertility as a consequence of copulating with several females, and thus, the mates of these males will seek extra-pair matings from lower-quality males (with fewer mates and lower immunocompetence) to ensure fertilization. These females will avoid laying infertile eggs, but have young with lower immunocompetence. In tree swallows, the high within-pair copulation rate, random distribution of the number of sires, and the lack of skew in fertilization success (Figs. 1 and 2) appears consistent with the fertility insurance hypothesis, but it seems more likely that the high rate of within-pair copulation is a response by males to multiple mating by the female, rather than a cause of it. For example, females might be able to avoid sperm depletion of their mate by less frequent within-pair copulation. Thus, the high frequency of within-pair copulation in tree swallows is most likely driven by males attempting to ensure their paternity (Venier and Robertson 1991; Crowe et al. 2009). Overall, it is difficult to exclude the male infertility hypothesis without experiments or direct measurements of sperm quality and quantity.

Another benefit of extra-pair mating and genetic polyandry, in particular, may be offspring that have greater immunocompetence or faster growth. Ultimately, this should result in greater offspring recruitment for females that produce broods sired by several different males. The only previous study in birds found no relationship between offspring recruitment and multiple paternity in coal tits, which have a moderate level of extra-pair paternity (28% extra-pair young in first broods) compared with tree swallows (Schmoll et al. 2007). Offspring recruitment is low in tree swallows (~1%), so we could not examine this in our study, but we did find that the mean PHA response of nestling tree swallows was stronger in broods that contained extra-pair young (particularly all extra-pair young) than in broods with just within-pair young (Fig. 3). On the other hand, we did not find a significantly stronger PHA response when there were more extra-pair sires in the brood or when we used a half-sib analysis of mixed paternity broods. O'Brien and Dawson (2007) also found no difference in the PHA response of extra- and within-pair young in a half-sib analysis of tree swallows in British Columbia, Canada, but they did find that extra-pair young grew their primary feathers faster than their within-pair half-sibs, and there was also a similar, but not significant ( $P=0.06$ ), pattern for growth in body mass. Nestling body mass and the length of both the ninth primary and tarsus are associated with recruitment in tree swallows (McCarty 2001). Although we found no effect of paternity on nestling growth rate (Table 2), the difference (0.026 g/day) between extra-pair ( $1.71\pm 0.051$  g/day) and within-pair ( $1.68\pm 0.051$  g/day) half-siblings (least squares means from half-sib analysis) was as large as that found in British Columbia ( $0.02\pm 0.01$  g/day) by O'Brien and Dawson (2007) with a sample size ( $N=30$  mixed paternity broods) slightly larger than ours ( $N=26$  for growth rate). Future studies should examine the potential environmental context of genetic benefits as it appears that extra-pair young outperform within-pair young under specific environmental conditions such as colder (Garvin et al. 2006) or warmer (Edler and Friedl 2008) temperatures, lower parasite infestations (O'Brien and Dawson 2007), or later in the season (Schmoll et al. 2005).

These studies indicate that there may be gene-by-environment interactions that obscure paternal genetic effects (e.g., good genes). Our results also highlight the potential effect of maternal or natal environment in influencing offspring quality and, subsequently, sexual selection. We found that females with extra-pair young had broods with higher average PHA responses than females without extra-pair young (Table 1, Fig. 3), but there was no evidence that this was related to paternal genes in a half-sib analysis. Thus, there appeared to be a natal (or maternal) environmental effect on immunity associated with extra-pair mating, but not a paternal genetic effect. It is possible that females increased

the PHA response of their young by investing more in their eggs when they engaged in extra-pair mating; however, extra-pair young did not come from heavier eggs in this study, as has been found in other species (Sprenger et al. 2008), and female tree swallows with extra-pair young did not provide more food to nestlings than females with all within-pair young (L. Whittingham and P. Dunn, unpubl. data). Females with extra-pair young could also have invested more in their eggs in terms of androgens (Gil et al. 1999; Navara et al. 2006) or immunoglobulins (Groothuis et al. 2005), which can influence nestling quality. These potential maternal effects need more study, but if they influence nestling immunity and extra-pair mating behavior is heritable, then there could be indirect selection for extra-pair mating because offspring produced in nests with extra-pair young (regardless of their paternity) will have stronger PHA responses and, possibly, greater survival. There are a few examples from arthropods in which multiple paternity was also associated with maternal or natal effects that increased offspring performance (Tregenza et al. 2003; Zeh and Zeh 2006). To the best of our knowledge, the only evidence of an association between maternal effects and mate choice in birds has been found in zebra finches (*Taeniopygia guttata*). Forstmeier et al. (2004) showed that females were more discriminating in their choice of mate if they had come from eggs that were laid early in a clutch. The potential for maternal effects to influence mate choice is widespread but relatively unstudied.

Although we found no evidence for a good genes effect of multiple paternity on offspring immunity or growth, there are a number of other possible genetic benefits of multiple paternity. For example, many studies suggest that females choose mates that are genetically dissimilar (reviewed in Tregenza and Wedell 1998), which is likely to increase the heterozygosity of individual offspring and their fitness (Mitton et al. 1993; Brown 1997; Amos et al. 2001). In an Ontario, Canada, population of tree swallows, Stapleton et al. (2007) found that the individual heterozygosity of extra-pair young was greater than that of their within-pair half-sibs. We did not find any effects of extra-pair paternity or multiple paternity on heterozygosity in this study, but this difference in results may be due to the larger sample of mixed paternity broods studied by Stapleton et al. ( $n=27$  vs. 70) and a partly different set of microsatellites used in each study (six of our seven loci overlapped with the 11 loci used by Stapleton et al. 2007). There also appeared to be large differences in heterozygosity between the 5 years studied by Stapleton et al. (2007), so we might only find an effect of heterozygosity in a longer-term study (difference in heterozygosity between extra- and within-pair young=0.006 in this study vs.  $\sim 0.06$  in Stapleton et al. 2007).

Although we found no effect of extra-pair (or multiple) paternity on heterozygosity of individuals, we did find it

increased diversity (alleles per locus and allelic richness) of the brood. These results suggest that female tree swallows gain more genetically diverse broods as the number of sires increases, and within the range studied here (up to six sires), genetic variation at the brood level continues to increase with the number of sires; it does not reach a threshold at which genetic diversity tapers off. The benefits of mating with many males remain unknown in birds, and theory (Yasui 1998) indicates that multiple mating for genetic diversity (at the brood level) is unlikely to evolve if fertilization patterns are random (i.e., there is no mate choice or sperm selection for more compatible or less related males). This does not appear to be true in tree swallows, as there is evidence that extra-pair sires are brighter (Bitton et al. 2007) and more genetically dissimilar (Stapleton et al. 2007) than the social mate. In fish and mammals, multiple paternity increases genetic diversity at the brood level in some cases (Garant et al. 2005; Cohas et al. 2007), but not in others (DiBattista et al. 2008; Lane et al. 2008). Given that multiple mating is unlikely to evolve because it increases brood diversity (Yasui 1998), it seems more likely that higher genetic diversity at the brood level is a non-selected by-product of female choice for extra-pair sires that are genetically dissimilar or more compatible (Stapleton et al. 2007).

In conclusion, there is increasing evidence in tree swallows of the influence of extra-pair mating on nestling quality (O'Brien and Dawson 2007; Stapleton et al. 2007), but the results may vary depending on environmental conditions. In this study, we did not find evidence for paternal genetic effects on offspring quality. However, female swallows produced broods sired by a large number of males, and at least in large broods, it appeared that females had more sires than would be expected by chance (Fig. 1), which suggests that females might actually seek a large number of mates. These results could also be a consequence of convenience polyandry, but other evidence suggests that there is little harassment of females, and even if it is occurring, this seems like an insufficient explanation for the unusually high levels of multiple paternity in tree swallows (relative to other birds). We think it is more likely that females mate with a large number of males to increase the likelihood of obtaining fertilizations from more genetically compatible sires (Stapleton et al. 2007). Ongoing studies are examining behavioral and ecological factors that influence the large number of different sires within broods of tree swallows.

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## References

- Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. *Proc R Soc Lond B* 268:2021–2027
- Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extra-pair copulation behavior in females. *Am Nat* 165:S26–S37
- Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* 397:151–154
- Bitton P-P, O'Brien EL, Dawson RD (2007) Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. *Anim Behav* 74:1777–1784
- Boag PT, Ratcliffe LM (2000) Genetics of avian mating systems. In: Apollonio M, Festa-Bianchet M, Mainardi D (eds) Vertebrate mating systems. World Scientific, Singapore, pp 307–332
- Brookfield JFY (1996) A simple method for estimating the null allele frequency from heterozygote deficiency. *Mol Ecol* 5:453–455
- Brown JL (1997) A theory of mate choice based on heterozygosity. *Behav Ecol* 8:60–65
- Christe P, de Lope F, González G, Saino N, Møller AP (2001) The influence of environmental conditions on immune response, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* 126:333–338
- Cohas A, Yoccoz NG, Allainé D (2007) Extra-pair paternity in alpine marmots, *Marmota marmota*: genetic quality and genetic diversity effects. *Behav Ecol Sociobiol* 61:1081–1092
- Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259–1267
- Crowe SA, Kleven O, Delmore KE, Laskemoen T, Nocera JJ, Lifjeld JT, Robertson RJ (2009) Paternity assurance through frequent copulations in a wild passerine with intense sperm competition. *Anim Behav* 77:183–187
- DiBattista JD, Feldheim KA, Gruber SH, Hendry AP (2008) Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks. *Mol Ecol* 17:783–795
- Dunn PO, Afton AD, Gloutney ML, Alisaukas RT (1999) Forced copulation results in few extrapair fertilizations in Ross's and lesser snow geese. *Anim Behav* 57:1071–1081
- Edler R, Friedl TWP (2008) Within-pair young are more immunocompetent than extrapair young in mixed-paternity broods of the red bishop. *Anim Behav* 75:391–401
- Firman RC, Simmons LW (2008) Polyandry facilitates postcopulatory inbreeding avoidance in house mice. *Evolution* 62:603–611
- Fisher DO, Double MC, Blomberg SP, Jennions MD, Cockburn A (2006) Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature* 444:89–92
- Forstmeier W, Coltman DW, Birkhead TR (2004) Maternal effects influence the sexual behavior of sons and daughters in the zebra finch. *Evolution* 58:2574–2583
- Garant D, Dodson JJ, Bernatchez L (2005) Offspring genetic diversity increases fitness of female Atlantic salmon (*Salmo salar*). *Behav Ecol Sociobiol* 57:240–244
- Garvin JC, Abroe B, Pedersen MC, Dunn PO, Whittingham LA (2006) Immune response of nestling warblers varies with extra-pair paternity and temperature. *Mol Ecol* 15:3833–3840
- Gil D, Graves J, Hazon N, Wells A (1999) Male attractiveness and differential testosterone investment in zebra finch eggs. *Science* 286:126–128
- Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol Ecol* 8:1231–1234
- Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. *J Hered* 86:485–486
- Griffith SC (2007) The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. *Am Nat* 169:274–281
- Griffiths R, Double M, Orr K, Dawson R (1998) A DNA test to sex most birds. *Mol Ecol* 7:1071–1076
- Groothuis TG, Eising CM, Dijkstra C, Müller W (2005) Balancing between costs and benefits of maternal hormone deposition in avian eggs. *Biol Letters* 1:78–81
- Hoffman JI, Forcada J, Trathan PN, Amos W (2007) Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* 445:912–914
- Hoogland JL (1998) Why do female Gunnison's prairie dogs copulate with more than one male? *Anim Behav* 55:351–359
- Hopper KR, Rosenheim JA, Prout T, Oppenheim SJ (2003) Within-generation bet hedging: a seductive explanation? *Oikos* 101:219–222
- Hussell D (1983) Age and plumage color in female tree swallows. *J Field Ornithol* 54:312–318
- Jamieson A (1994) The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. *Anim Gen* 25:37–44
- Jeffreys AJ, Allen M, Hagelberg E, Sonnberg A (1992) Identification of the skeletal remains of Josef Mengele by DNA analysis. *Forensic Sci Int* 56:65–76
- Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biol Rev* 75:21–64
- Johnsen A, Andersen V, Sunding C, Lifjeld JT (2000) Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* 406:296–299
- Lane JE, Boutin S, Gunn MR, Slate J, Coltman DW (2008) Female multiple mating and paternity in free-ranging North American red squirrels. *Anim Behav* 75:1927–1937
- Lee PLM, Hays GC (2004) Polyandry in a marine turtle: females make the best of a bad job. *Proc Nat Acad Sci USA* 101:6530–6535
- Lifjeld J, Robertson R (1992) Female control of extra-pair fertilizations in tree swallows. *Behav Ecol Sociobiol* 31:89–96
- Lifjeld JT, Dunn PO, Whittingham LA (2002) Short-term fluctuations in cellular immunity of tree swallows feeding nestlings. *Oecologia* 130:185–190
- Lochmiller RL, Vestey MR, Boren JC (1993) Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk* 110:503–510
- Madsen T, Shine R, Loman J, Hakansson T (1992) Why do female adders copulate so frequently? *Nature* 355:440–441
- Mäkinen T, Panova M, André C (2007) High levels of multiple paternity in *Littorina saxatilis*: hedging the bets? *J Hered* 98:705–711
- Maklakov AA, Lubin Y (2004) Sexual conflict over mating in a spider: increased fecundity does not compensate for the costs of polyandry. *Evolution* 58:1135–1140
- Martin LB, Han P, Lewittes J, Kuhlman JR, Klasing KC, Wikelski M (2006) Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoeological technique. *Func Ecol* 20:290–299

- McCarty JP (2001) Variation in growth of nestling tree swallows across multiple temporal and spatial scales. *Auk* 118:176–190
- McKinney F, Everts S (1998) Sexual coercion in waterfowl and other birds. In: Parker P, Burley N (eds) *Avian reproductive tactics: female and male perspectives*, vol 49. American Ornithologists' Union, Washington, D.C., pp 163–195
- Meek SB, Robertson RJ, Boag PT (1994) Extrapair paternity and intraspecific brood parasitism in eastern bluebirds revealed by DNA fingerprinting. *Auk* 111:739–744
- Mitton JB, Schuster WSF, Cothran EG, DeFries JC (1993) Correlation between the individual heterozygosity of parents and their offspring. *Heredity* 71:59–63
- Navara KJ, Hill GE, Mendona MT (2006) Yolk androgen deposition as a compensatory strategy. *Behav Ecol Sociobiol* 60:392–398
- Neff BD, Pitcher TE, Ramnarine IW (2008) Inter-population variation in multiple paternity and reproductive skew in the guppy. *Mol Ecol* 17:2975–2984
- O'Brien EL, Dawson RD (2007) Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine. *Behav Ecol Sociobiol* 61:775–782
- Olsson M, Gullberg A, Tegelström H, Madsen T, Shine R (1994) Can female adders multiply? *Nature* 369:528
- Otter K, Ratcliffe L, Boag PT (1998) Do female black-capped chickadees prefer high-ranking males as extra-pair partners? *Behav Ecol Sociobiol* 43:25–36
- Parker G (1990) Sperm competition games: raffles and roles. *Proc R Soc Lond B* 242:120–126
- Petit R, el Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv Biol* 12:844–855
- Poirier NE, Whittingham LA, Dunn PO (2004) Males achieve greater reproductive success through multiple broods than through extrapair mating in house wrens. *Anim Behav* 67:1109–1116
- Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J, Burke T (2000) Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol Ecol* 9:2225–2229
- Robertson RJ, Rendell WB (2001) A long-term study of reproductive performance in tree swallows: the influence of age and senescence on output. *J Anim Ecol* 70:1014–1031
- Robertson RJ, Stutchbury BJ, Cohen RR (1992) Tree Swallow. In: Poole A, Stettenheim P, Gill F (eds) *The Birds of North America*, no 11. Academy of Natural Sciences, Philadelphia, pp 1–28
- SAS Institute (2003) JMP 5.0.1 User's guide. SAS Institute, Cary, NC
- Schmoll T, Dietrich V, Winkel W, Epplen JT, Schurr F, Lubjuhn T (2005) Paternal genetic effects on offspring fitness are context dependent within the extrapair mating system of a socially monogamous passerine. *Evolution* 59:645–657
- Schmoll T, Schurr FM, Winkel W, Epplen JT, Lubjuhn T (2007) Polyandry in coal tits *Parus ater*: fitness consequences of putting eggs into multiple genetic baskets. *J Evol Biol* 20:1115–1125
- Simmons LW (2005) The evolution of polyandry: sperm competition, sperm selection and offspring viability. *Ann Rev Ecol Evol Syst* 36:125–146
- Smits JE, Bortolotti GR, Tella JL (1999) Simplifying the phytohemagglutinin skin testing technique in studies of avian immunocompetence. *Func Ecol* 13:567–572
- Sprenger D, Anthes N, Michiels N (2008) Multiple mating affects offspring size in the opisthobranch *Chelidonura sandrana*. *Mar Biol* 153:891–897
- Stapleton M, Kleven O, Lifjeld J, Robertson R (2007) Female tree swallows (*Tachycineta bicolor*) increase offspring heterozygosity through extrapair mating. *Behav Ecol Sociobiol* 61:1725–1733
- Tregenza T, Wedell N (1998) Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* 52:1726–1730
- Tregenza T, Wedell N, Hosken DJ, Ward PI (2003) Maternal effects on offspring depend on female mating pattern and offspring environment in yellow dung flies. *Evolution* 57:297–304
- Venier LA, Robertson RJ (1991) Copulation behaviour of the tree swallow, *Tachycineta bicolor*: paternity assurance in the presence of sperm competition. *Anim Behav* 42:939–948
- Venier LA, Dunn PO, Lifjeld JT, Robertson RJ (1993) Behavioural patterns of extra-pair copulation in tree swallows. *Anim Behav* 45:412–415
- Whitlock MC, Schluter D (2009) *The analysis of biological data*. Roberts, Greenwood Village
- Whittingham L, Dunn P (2000) Offspring sex ratios in tree swallows: females in better condition produce more sons. *Mol Ecol* 9:1123–1129
- Whittingham LA, Dunn PO, Stapleton MK (2006) Repeatability of extra-pair mating in tree swallows. *Mol Ecol* 15:841–849
- Whittingham LA, Dunn PO, Lifjeld JT (2007) Egg mass influences nestling quality in tree swallows, but there is no differential allocation in relation to laying order or sex. *Condor* 109:585–594
- Yasui Y (1998) The 'genetic benefits' of female multiple mating reconsidered. *Trends Ecol Evol* 13:246
- Yasui Y (2001) Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. *Ecol Res* 16:605–616
- Zach R (1982) Hatching asynchrony, egg size, growth, and fledging in tree swallows. *Auk* 99:695–700
- Zeh JA, Zeh DW (1996) The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proc R Soc Lond B* 263:1711–1717
- Zeh JA, Zeh DW (2006) Outbred embryos rescue inbred half-siblings in mixed-paternity broods of live-bearing females. *Nature* 439:201–203