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Original Article

Heterozygosity and fitness benefits of extrapair mate choice in White-rumped Swallows (*Tachycineta leucorrhoa*)

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Female birds that engage in extrapair mating may choose extrapair mates that are genetically compatible, increasing their fitness through genetic benefits, such as increased heterozygosity, to their offspring; or choose mates that are heterozygous at one or more loci. Here, we describe the extrapair mating system, explore the fitness benefits of extrapair mating and test the heterozygosity hypothesis in White-rumped Swallows (*Tachycineta leucorrhoa*) breeding in Argentina using a panel of microsatellite loci. Extrapair offspring accounted for 56% of the nestlings and 77% of the broods in our population. Within broods, 1–4 males fathered extrapair offspring, and in 29% of nests, all offspring were from extrapair sires. We found that broods with extrapair offspring fledged overall more young than broods with no extrapair offspring but that the young that died were more heterozygous than the ones that fledged. Although extrapair offspring had a higher probability of surviving than within-pair offspring, these 2 groups did not differ in their level of heterozygosity. Neither the heterozygosity of the social mate nor the genetic similarity of the social pair predicted the presence of extrapair young. Instead, females chose social mates that were significantly less genetically similar to them. Our results do not support the heterozygosity hypothesis and contradict 2 of its main predictions.
Key words: extrapair paternity, fitness benefits, heterozygosity, mating systems, *Tachycineta*. *[Behav Ecol 22:1178–1186 (2011)]*

INTRODUCTION

Since the first application of molecular techniques to studies of parentage in birds in the late 1980's (Burke and Bruford 1987) much progress has been made in our understanding of avian mating systems and their variation. Matings outside the pair bond may clearly be advantageous for males by directly increasing the number of offspring sired in a given season (reviewed in Birkhead and Møller 1992). Fitness benefits of extrapair mating for females, however, have been more difficult to identify due to the indirect nature of most such benefits (Jennions and Petrie 2000; Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007).

By engaging in extrapair copulations, females can modify their choice of a partner after securing a social mate, resulting in a mixed reproductive strategy. It has been proposed that females that choose their mates can obtain 2 types of benefits: 1) direct benefits in the form of nesting sites, access to resources, parental care, etc.; or 2) indirect benefits through an increase in their offspring's genetic quality (see review in Andersson 1994). The adaptive value of extrapair behavior has often been associated with a gain in indirect benefits to females (reviewed in Jennions and Petrie 2000). By mating multiply with high-quality males, females can accrue good genes for their offspring (i.e., Fisher 1915; Zahavi 1975). Petrie and Lipsitch (1994) suggested that if it pays females to seek indirect genetic benefits through extrapair matings, then females should mate with more than one male only when there is sufficient genetic variation among males. However, if there is a preference for a particular heritable male trait among females, the genetic variability on this trait in the population soon will be lost (Tregenza and Wedell 2000)—directional sexual selection will decrease genetic variability on the trait under selection to a point where it does not pay to be choosy on the basis of such trait.

Zeh JA and Zeh DW (1996, 1997) and Brown (1997) proposed an alternative explanation for the indirect-benefits female choice. Their hypotheses indicate that genetic quality is not gained through a heritable trait, but it is rather a result of heterozygosity at one or more loci. The hypothesis on genetic compatibility proposed by Zeh JA and Zeh DW (1996, 1997) states that offspring viability and female fitness increase when females mate with males whose genomes best complement their own. Therefore, polyandry might have evolved as a mechanism to avoid male–female genome incompatibilities (usually referred to as the "genetic compatibility hypothesis"). Brown's (1997) proposed hypothesis is based on 2 alternative ideas: 1) females will favor a choice of males such that their offspring will be heterozygous at some or many loci, which should result in

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increased offspring fitness with increased heterozygosity (there is no single best male/trait for the female population, Brown 1997; Jennions 1997); and 2) even when females might choose a best male, the male's superiority may be because he is heterozygous at one or more loci, which is a nonheritable trait ("heterozygosity theory," Brown 1997; Hansson and Westerberg 2002). Although these hypotheses remain controversial (Wetzel and Westneat 2009), the underlying assumption is that offspring resulting from genetically compatible matings are less likely to suffer the negative effects of inbreeding (Brown 1997). Because of the similarities in some of the predictions made by these 2 hypotheses, we will combine both ideas and refer to them as the "heterozygosity hypothesis."

In this paper, we provide the first detailed information on extrapair paternity (EPP) rates for a south temperate breeder, the White-rumped Swallow (*Tachycineta leucorrhoa*), and examine the effects of genetic compatibility and heterozygosity, as measured by comparisons of microsatellite genotypes, on offspring fitness and its relation to EPP. We tested the following predictions derived from the heterozygosity hypothesis of EPP (modified from Wetzel and Westneat 2009): 1) offspring heterozygosity is negatively related to the genetic similarity of their sires, 2) extrapair offspring are more heterozygous than within-pair young, 3) more heterozygous offspring have a higher survival rate, 4) genetic similarity of the social breeding pair positively predicts EPP status at the nest, 5) extrapair males are less genetically similar to the female than the within-pair males are to the female, and 6) the extrapair male is more heterozygous than the within-pair male.

MATERIALS AND METHODS

Field methods

Tachycineta swallows are secondary cavity nesters, and most species readily breed in artificial nest-boxes placed in study colonies. The White-rumped Swallow's breeding distribution ranges from Buenos Aires Province (Argentina) in the south to northern Bolivia and southern Brazil in the north (Turner and Rose 1989). Our work was conducted at a breeding colony in Chascomús, Buenos Aires (lat 35°34′S, long 58°01′W), where 126 nest-boxes were spaced at 25–35 m distances. This colony has been active since 2002, and about 65% of the boxes in the colony are being used each year (Ferretti V, personal observations). We studied this population during 2 consecutive breeding seasons (2006–2007).

White-rumped Swallows are socially monogamous, and both males and females contribute to the care of the young during the breeding season (Bulit et al. 2008). At our colony, some pairs raise 2 broods within a breeding season (Massoni et al. 2007), but double brooding is absent or rare (<2%) at nearby colonies at similar latitudes (Ferretti V, unpublished data). To assess the fate of nests, boxes were checked every other day from egg laying until nestlings were 15 days old. For each breeding attempt, we recorded lay date (i.e., date of the first-laid egg), clutch size, and brood size. Clutches were considered complete when their size did not change for at least 2 days. We did not check nests with nestlings older than 15 days to avoid premature fledging of the young; we did, however, continue to monitor nests from a distance to ensure that they remained active until fledging. The status of social mates was confirmed at each nest by observations that the social mate was the only male provisioning the focal nest. Once parental activity ceased at the nest—usually after day 20—we checked for dead nestlings inside the box and calculated the number of fledglings as the number of nestlings seen at the nest on day 15 minus the number of dead nestlings inside the box; after nestling day 10, parents generally do not remove dead chicks, and the resulting carcasses can later be found at the bottom of the nest cup once all the other chicks have fledged (Ferretti V, personal observations). Nests were considered depredated if eggs or young disappeared when they were too young to fledge.

Genetic sample collection

For every nesting attempt, we captured both adult breeders while they were inside the nest-boxes using box traps (see http://golondrinas.cornell.edu for details on boxes and traps). Captured adults were measured, bled, and banded with aluminum bands with unique numbers. Females were most often captured during incubation and recaptured when feeding nestlings. Males were captured while feeding nestlings and were additionally marked with nontoxic colored markers at the time of banding for visual identification in a simultaneous study on parental visitation rates (see Bulit et al. 2008). When nestlings were 7–9 days old, we banded them with numbered aluminum bands and took a blood sample from each. We took 20–70 µl of blood from both adults and nestlings, collected it using a heparinized capillary tube via brachial venipuncture, and then stored whole blood in Queen's lysis buffer (Seutin et al. 1991). When nestlings were found dead in the nest before they were banded and bled (before day 7–9), we collected a sample from their pectoral muscle and stored it in 96% ethanol for further genetic analyses.

Microsatellite amplification for paternity exclusions and assignments

We extracted DNA from blood and muscle samples using DNA purification kits by Eppendorf (Perfect gDNA blood mini isolation kit, Hamburg, Germany) and Qiagen (DNeasy blood and tissue kit, Valencia, CA). Extracted DNA was diluted 1:10 in ultra purified H_2O and then amplified at 12 highly polymorphic microsatellite loci (Table 1) following the conditions from Makarewich et al. (2009). We amplified multiple loci in multiplexed polymerase chain reactions (PCRs); this allowed us to score all 12 loci with only 3 PCR reactions per individual. The combination of primers used in each of these multiplexed reactions was selected so as to avoid PCR product overlap by their fragment sizes as well as by using unique fluorescent dyes. PCRs were performed in 10 µl final volumes. Each of the 3 mixes used 10–100 ng DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 3.25 mM $MgCl_2$, 200 µM dNTPs

Table 1
Microsatellite loci used in this study and their characteristics

Locus	Allele size range	N	N_A	H_O	H_E	PCR Mix
Tabi1	306–347	110	16	0.676	0.883	1
Tabi4	261–300	110	16	0.802	0.854	1
Tle16	253–268	110	10	0.604	0.647	1
Tle17	228–242	110	11	0.811	0.845	1
Tle19	154–173	110	12	0.811	0.803	1
Tle4	204–298	110	30	0.919	0.934	2
Tle8	231–250	110	15	0.892	0.886	2
Tal7	338–481	110	49	0.946	0.969	2
Tal11	211–220	110	8	0.766	0.797	3
Tal6	335–362	110	14	0.901	0.862	3
Tal8	267–403	110	38	0.964	0.949	3
Tle21	166–178	110	10	0.676	0.686	3

N: number of unrelated individuals genotyped, N_A: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, and PCR Mix: primers used in the same multiplexed PCR reaction, equal numbers represent primers used in the same multiplex PCR reaction.

(Invitrogen, Carlsbad, CA), 0.25 U Jumpstart *Taq* polymerase (Sigma-Aldrich, St Louis, MO), the specified mix of forward and reverse primers, and H_2O to bring the final volume to 10 µl. Mix 1 contained 1 pM Tle19, 2.4 pM Tle17, 1 pM Tle16, 4.8 pM Tabi4, and 1.2 pM Tabi1 forward and reverse primers. Mix 2 contained 2.4 pM Tle4, 1.2 pM Tle8, and 2.4 pM Tal7 forward and reverse primers. Mix 3 contained 1.2 pM Tle21, 1.8 pM Tal11, 3.6 pM Tal8, and 1.6 pM Tal6 forward and reverse primers.

PCRs were performed in a DYAD thermal cycler (Bio-Rad, Hercules, CA). Cycling profiles for mixes 1 and 2 followed 1 incubation cycle of 95 °C for 2 min; 35 cycles of 50 s at 95 °C, 1 min at an annealing temperature of 56 °C, and an extension time of 1 min at 72 °C; these 35 cycles were followed by a final extension phase of 30 min at 72 °C. Mix 3 PCR cycle was the same as for 1 and 2 with the exception that the annealing temperature used was 58 °C. PCR products were then genotyped on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA), and the sizes of the microsatellite alleles estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems).

Allele frequencies and observed (H_O) and expected heterozygosity (H_E) at each locus were generated using the program Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). Paternity exclusions and assignments were performed with the microsatellite profiles generated by the program GeneMapper using Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007), a likelihood-based method. This program calculates the probability of paternal exclusion when one parent is known (in our case the mother) for each locus. The combined exclusion probability for the 12 loci used was 0.9999.

We analyzed only families for which we had complete information (e.g., DNA sample from the social male, social female, and nestlings). We first compared the nestlings' genotypes with the genotype of the adult female attending their nest (putative mother). Most nestlings shared an allele in each of the 12 loci with their putative mother, as expected. Some nestlings (42 of a total of 342 nestlings) did not match the social female at 1 of the 12 loci; we regard these nestlings as offspring of their putative mothers and assume this single-locus allele difference a result of rare mutations or genotyping errors (Fernando et al. 2001). No nestlings mismatched their putative mother at 2 or more loci. The nestlings' genotypes were then compared with those of their putative father. If nestlings mismatched the social father's genotype at 2 or more loci, we considered them extrapair young. Additionally, we compared the paternal alleles of the nestlings with the alleles of all the males genotyped in our population to assign potential extrapair sires while recognizing that not all potential sires were sampled.

Measures of reproductive success

We used clutch size and number of young fledged as measures of reproductive performance. We did not consider nests that failed during incubation in our comparisons of number of young fledged between groups (for information on hatching and incubation success, see Massoni et al. 2007). Only nests that hatched young could be sampled for paternity analyses, and hence for these nests, we have measures of both clutch size and number of young fledged. We used a mixed linear model (Restricted Maximum Likelihood [REML] estimation) with year as a random factor with a compound symmetric covariance structure to compare observed differences in clutch size between nests with different paternity status: Nests with one or more extrapair young (EPN) sired by females that engaged in extrapair behavior versus nests with no extrapair young (WPN) sired by females that presumably did not engage in extrapair behavior. Likewise, we also used a mixed linear model (REML estimation) with year as a random factor with a compound symmetric covariance structure to compare number of young fledged between nests with different paternity status. For this second comparison, we subdivided nests with extrapair young into 2 categories, resulting in 3 groups: nests with all extrapair young in them (ALL EPY), nests with all within-pair young in them (ALL WPY), and broods of mixed paternity (MIXED). We did this to be able to identify differences in fledging success due to paternity status of the nestlings (i.e., their genetic buildup) from those differences due to the parental care quality of the attending adults. If there is a fitness advantage in extrapair young, nests of mixed paternity should fledge on average more young than those that fledge from nests with all within-pair young but fewer than the number of young that fledge from nests with all extrapair offspring. In contrast, if fledging success is mainly influenced by parental care quality, and it is in turn associated with extrapair behavior, nests with mixed paternity and those with all extrapair young should fledge a similar number of nestlings, higher on average than those that fledge from nests with all within-pair offspring. We also performed these analyses using percent of extrapair nestlings within a brood as a continuous variable. The results of these tests were essentially similar to those using the categories aforementioned and are therefore not included in the paper.

We examined the probability that extrapair nestlings (EPY) would be equally likely to fledge than within-pair nestlings (WPY) with a Fisher's Exact test. We did this for all nests sampled, and we repeated this analysis only considering those nests with broods of mixed paternity. We also examined the nestlings' probabilities of fledging according to the extrapair status of the nest where they were raised (i.e., nests with all within-pair young, nests with mixed broods, and nests with all extrapair young) using a chi-square test.

Measures of heterozygosity and genetic compatibility

We used standardized heterozygosity (H_{ST}), based on the mean $H_{O,}$ as a measure of inbreeding status of an individual (Coltman et al. 1999). This measure of heterozygosity takes into account the proportion of heterozygous loci divided by the mean observed heterozygosity; it is highly conservative and performs better than other measures when there is allele dropout or when individuals are genotyped at different numbers of loci (Coulon 2010).

We calculated relatedness between the breeding adults using the program KINGROUP v2 (Konovalov et al. 2004) and used this value as a measure of genetic similarity of the pair. The mean genetic similarity of our sampled adult population was −0.0089 (expected value is 0, Stapleton et al. 2007). We examined the correlation between offspring heterozygosity and genetic similarity of the genetic parents by using Pearson's coefficient (for EPY, we used only those cases for which we were able to assign a genetic father to the offspring). We used a mixed linear model (REML estimation), with year and nest as random effects, with a compound symmetric covariance structure, to control for maternal and seasonal effects, to compare H_{ST} of EPY and WPY as well as H_{ST} of offspring that died and those that survived. We also compared H_{ST} of nestlings from different nest types (i.e., nests with all EPY, nests with all WPY, and mixed paternity broods) using a mixed linear model (REML estimation) with year and nest as random effects with compound symmetric covariance structure. We used binary logistic regressions to test for the ability of the social male's H_{ST} and genetic similarity between the social breeding pair to explain EPP status (presence or absence of extrapair young at any one nest). For those cases in which we could identify the

extrapair sire, we compared the genetic similarity of the social dyad (i.e., genetic similarity between the female and her social mate) with that of the extrapair dyad (i.e., female with the extrapair male) with a paired *t*-test. We also compared H_{ST} of the social male with that of the extrapair male using a paired *t*-test. Statistical analyses were performed using SAS (version 9.2), JMP (version 8.0.1), and SPSS (version 14).

We conducted randomization tests to test for the genetic similarity between the female and within-pair male, and the female and extrapair male, because of statistical biases that can be generated when comparing the extrapair and within-pair male's genetic similarities to the female, against each other (Wetzel and Westneat 2009). These tests allowed us to compare the observed genetic similarities against a random distribution of males in the population. We followed the methods used in Fossøy et al. (2007). In sum, for the randomization test for within-pair males, we used the genetic similarity between all males breeding in the population and all females. For the randomization test for extrapair males, we considered the genetic similarity of all males in the population with all breeding females in the population but excluded their within-pair partners. We conducted both randomization tests using the software Resampling Stats (version 4.0) and iterated each test 10 000 times.

RESULTS

Reproductive success and EPP

We captured a total of 171 adults (87 females and 84 males). In both years combined, we captured 90% of the breeding females in our population. The percentage of males captured in the colony was 75%. As some of the pairs in our population raise 2 broods within a breeding season, we present 2 sets of results in Table 2: One summary that takes into account all the nests sampled, and one in which only first broods are considered. In total, we studied 78 broods of 55 breeding pairs and genotyped 342 nestlings (Table 2). In addition, to search for putative fathers, we genotyped 22 resident males that were captured in our study area but whose nests were excluded from the paternity analyses (i.e., experimental nests, etc.).

We found the total rate of EPP in our population to be 77% for broods with extrapair young and 56% for extrapair nestlings (Table 2). We did not find differences in rates of EPP across years ($\chi^2 = 0.894$, $P = 0.344$), thus we present the data for both years combined in a single estimate. Twenty-three of the extrapair nests had all nestlings sired by extrapair males (29% broods, Table 2). To confirm these were not misidentifications of the social male nor laboratory sample switches, we first regenotyped all individuals in these family groups and then cross-checked our information with that of the video recordings of parents feeding nestlings used in a concurrent study on parental care.

Table 2
Summary of EPP rates for White-rumped Swallows

	All nests	First broods
Total number of nests	78 nests	55 nests
Total number of nestlings	342 nestlings	246 nestlings
Nests with extrapair offspring	60 nests (77%)	43 nests (78%)
Nests with all extrapair offspring	23 nests (29%)[a]	17 nests (31%)[a]
Extrapair offspring	193 nestlings (56%)	136 nestlings (55%)

[a] The percentage of nests containing all extrapair offspring is based on the total number of nests sampled in the population not just those with extrapair young.

In all 23 cases, we derived the same genotypes for the individuals in these families, and the male bringing food to the nestlings was the one with the markings added at the time of capture/sampling. Within this group, we identified 4 nests (5% of the total broods sampled) for which the social male, despite having lost all the paternity at his own nest, sired offspring at neighboring nests. Other males in this group might have also sired offspring in nests that we did not sample.

Of the 342 nestlings sampled, 193 were extrapair offspring (56%, Table 2). We were able to identify the biological father for 90 of the 193 extrapair nestlings (46.63%) with high probability. Within broods, one to several males sired extrapair young—from our assignments, we were able to detect up to 4 extrapair males, although there could have been more in the frequent cases where we could not assign all biological fathers of the extrapair offspring.

Differences in clutch size for nests with and without extrapair young were not significant ($F_{1,75} = 1.92$, $P = 0.17$, power = 0.903, variance due to random effect = 0.004, Figure 1A), but nests with at least one EPP offspring fledged overall more young than did those without EPP offspring ($F_{2,74} = 3.57$, $P = 0.03$, variance due to random effect = 0.026, Figure 1B). We conducted post hoc comparisons to identify differences between categories and found that ALL EPY nests significantly fledged more young than ALL WPY nests (Student's *t*-test, $P = 0.009$), but we did not find differences between the other pairs (MIXED-ALL WPY Student's *t*-test, $P = 0.089$; ALL EPY-MIXED Student's *t*-test, $P = 0.215$). EPY had a greater probability of surviving than did WPY when all nests were considered (Fisher's Exact test, $P < 0.001$, $N = 342$), but when only nests with mixed broods were used in the analyses, we failed to find a significant difference in probability of survival with respect to EPP status (Fisher's Exact test, $P = 0.591$, $N = 153$), suggesting that the difference detected when all nests were considered was more likely a consequence of the quality of the social parents as parental care providers rather than the extrapair status of individual offspring per se.

The probability of fledging differed among groups and depended on the status of the nest where the nestling was raised. Nestlings from nests with all within-pair young had the lowest probability of surviving, and young in nests with all extrapair offspring had the highest per-chick probability of surviving ($\chi^2 = 27.591$, $P < 0.001$, $N = 342$).Figure 2), but offspring that did not survive had a higher H_{ST} than did those that fledged (Prediction 3 Introduction, $F_{1,268} = 4.10$, $P = 0.04$, variance due to random effects = 0.003, Figure 3). We found similar patterns when only nests of mixed paternity were considered for the analysis ($F_{1,135} = 0.0004$, $P = 0.98$, variance due to random effects = 0.005; $F_{1,64} = 4.02$, $P = 0.05$, variance due to random effects = 0.005, respectively). We did not find significant differences in the level of H_{ST} of the nestlings in broods of different paternity status ($F_{2,267} = 0.87$, $P = 0.42$, power = 0.327, variance due to random effects = 0.003, Figure 4).

For the analysis of heterozygosity, we sampled pairs only once (i.e., we did not use the information on the second broods of pairs that renested), and we included only individuals that were typed at 8 or more loci. This resulted in a total sample of 65 nests. We did not find within-pair male H_{ST} to be a good predictor of EPP status ($\chi^2 = 1.79$, $P = 0.18$) nor did genetic similarity between the members of the social pair predict EPP status (Prediction 4 Introduction, $\chi^2 = 0.27$, $P = 0.61$).

We were able to compare the genetic similarity of the social pair with the genetic similarity of the female and extrapair male for those cases in which we could identify extrapair sires. Extrapair males sometimes sired more than one nestling in a single brood and some sired nestlings in different broods.

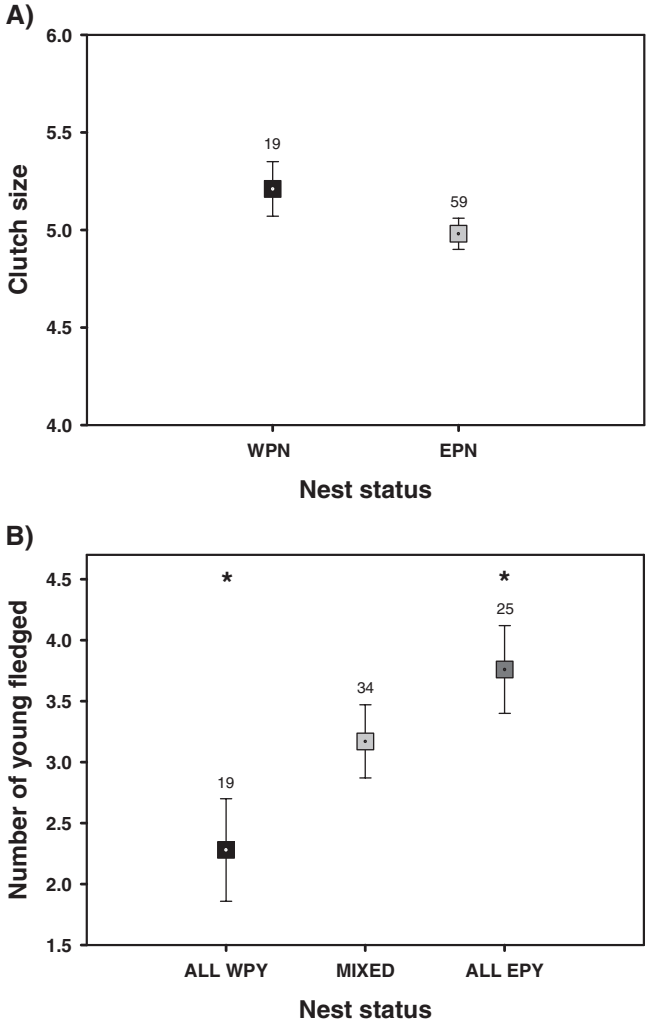

Figure 1
Reproductive performance differences between nests with extrapair offspring (EPN) and nests with only within-pair offspring (WPN). The *y* axis represents clutch size (A), and number of young fledged (B). Sample sizes for each group are shown above the boxes. Asterisk represents a significant difference ($\alpha = 0.05$). Results presented as mean ± standard errors. Actual values of means ± standard errors and upper–lower 95% confidence intervals for each group were: (A) Mean clutch size EPN 4.98 ± 0.08, 4.82–5.14; WPN: 5.21 ± 0.14, 4.93–5.49, (B) Mean number of nestlings fledged, nests with all extrapair offspring: ALL EPY 3.76 ± 0.36, 3.06–4.46; nests with all within-pair offspring: ALL WPY 2.16 ± 0.40, 1.35–2.96; and nests with offspring of mixed paternity: MIXED 3.26 ± 0.30, 2.66–3.87.

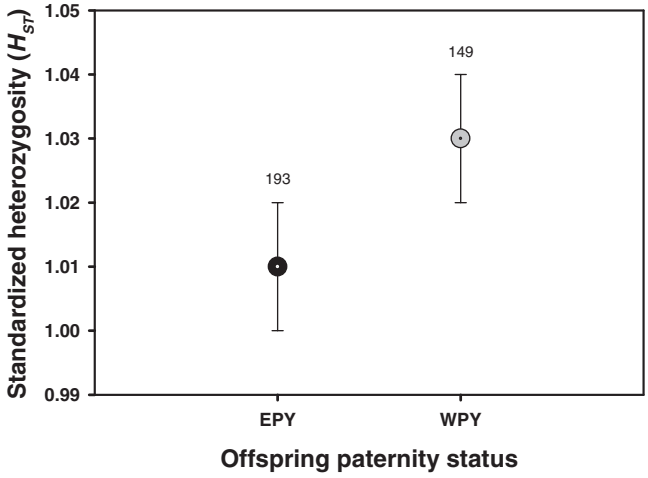

Figure 2
Standardized heterozygosity for extrapair and within-pair nestlings. The *y* axis represents the standardized heterozygosity, and the *x* axis represents the offspring paternity status. Sample sizes for each group are shown above the circles. Results are presented as mean ± standard errors. Actual values of means ± standard errors and upper–lower 95% confidence intervals for each group were: EPY: 1.01 ± 0.01, 0.99–1.03; WPY: 1.03 ± 0.01, 1.00–1.05. H_{ST}: Standardized heterozygosity, EPY: extrapair young, and WPY: within-pair young.

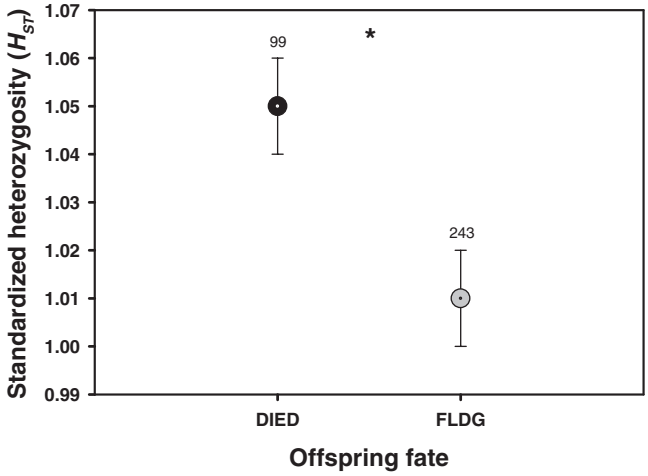

Figure 3
Standardized heterozygosity for nestlings that died and those that fledged. The *y* axis represents the standardized heterozygosity, and the *x* axis represents the offspring's fate. Sample sizes for each group are shown above the circles. Results are presented as mean ± standard errors. Actual values of means ± standard errors and upper–lower 95% confidence intervals for each group were: DIED: 1.05 ± 0.01, 1.03–1.08; FLDG: 1.01 ± 0.01, 0.99–1.02. H_{ST}: Standardized heterozygosity, DIED: nestlings that died in the nest, and FLDG: nestlings that survived. Asterisk represents a significant difference ($\alpha = 0.05$).

Thus, for those that sired multiple nestlings with one female, we only used one case for that nest, but for those that sired nestlings in different broods, we took a measure of genetic similarity of that male with each of the females with whom he sired offspring. Whenever possible, we formed "dyads" of all female's known matings (female with extrapair male vs. female with social mate) for comparison. We found the genetic similarity between the female and her social male to be smaller (i.e., the female and the social male were less genetically similar) than the genetic similarity of the female with her extrapair partners (Prediction 5 Introduction, paired *t*-test, $t = 3.87$, $P < 0.001$, $N = 34$). However, there was no significant correlation between the measures of genetic similarity within the dyads (Pearson's $r = -0.08$, $P = 0.68$, power = 0.364, $N = 34$, Figure 5A)—that is, females that were more genetically similar to their social males did not consistently mate with extrapair partners that were less genetically similar to them, nor vice versa, suggesting no relationship between the female's choice of a social mate and her subsequent choice of an extrapair mate. In addition, H_{ST} of the extrapair male was not significantly different than that of the social male (Prediction 6 Introduction, paired *t*-test, $t = 1.44$,

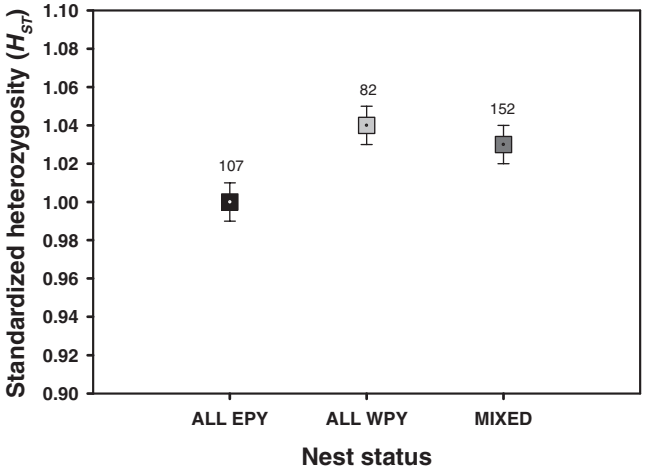

Figure 4
Standardized heterozygosity for nestlings in different nests. The *y* axis represents the standardized heterozygosity, and the *x* axis represents the nest status in relation to the paternity of the brood. Sample sizes for each group are shown above the boxes. Results are presented as mean ± standard errors. Actual values of means ± standard errors and upper–lower 95% confidence intervals for each group were: ALL EPY: 1.00 ± 0.01, 0.98–1.03; ALL WPY: 1.04 ± 0.01, 1.00–1.07; and MIXED: 1.03 ± 0.01, 1.00–1.05. H_{st}: Standardized heterozygosity, ALL EPY: broods with all extrapair offspring, ALL WPY: broods with all within-pair offspring, and MIXED: broods of mixed paternity. Results presented as mean ± standard errors.

$P = 0.16$), and there was no significant correlation in H_{ST} between the males in the dyads (Pearson's $r = 0.12$, $P = 0.49$, power = 0.173, $N = 34$, Figure 5B).

We used randomization tests to compare the genetic similarity between the within-pair male and the female against that of all males available in the population, as well as the genetic similarity of the extrapair male to the female in relation to all potential extrapair partners in the study plot. We found the social males to be less genetically similar to the female than expected from a random choice of mates ($P < 0.01$); but the genetic similarity between the extrapair partners and the females did not differ significantly from random choice ($P = 0.28$, Table 3).

DISCUSSION

EPP and reproductive success

Like most passerine birds, White-rumped Swallows engage in extrapair mating. These swallows, however, are unusual in that their rate of EPP is far higher than that of most socially monogamous songbirds. We found that 77% of nests had extrapair young and more than half of the total offspring (56%) were sired by extrapair males (Table 2), whereas across other passerines, the corresponding means are 18.7% of broods and 11.1% of offspring (Griffith et al. 2002). As mentioned in the results section, we were able to identify the biological father in 46% of the extrapair young sampled. Adult White-rumped Swallows show a promiscuous behavior in that within broods, offspring are frequently sired by more than 2 males, and nearly 29% of the nests in our population had all nestlings sired by one to several extrapair males (Table 2). The proportion of nests in the population where the socially attendant male lost all paternity appears higher than that observed in the congener Tree Swallow (*Tachycineta bicolor*, 29% of nests vs. 9.25–18.4% of nests in Tree Swallow, Kempenaers et al. 1999; Whittingham and Dunn 2001), which also shows extremely high rates of EPP. However, a few of these White-rumped Swallow males that lost paternity at their own nests

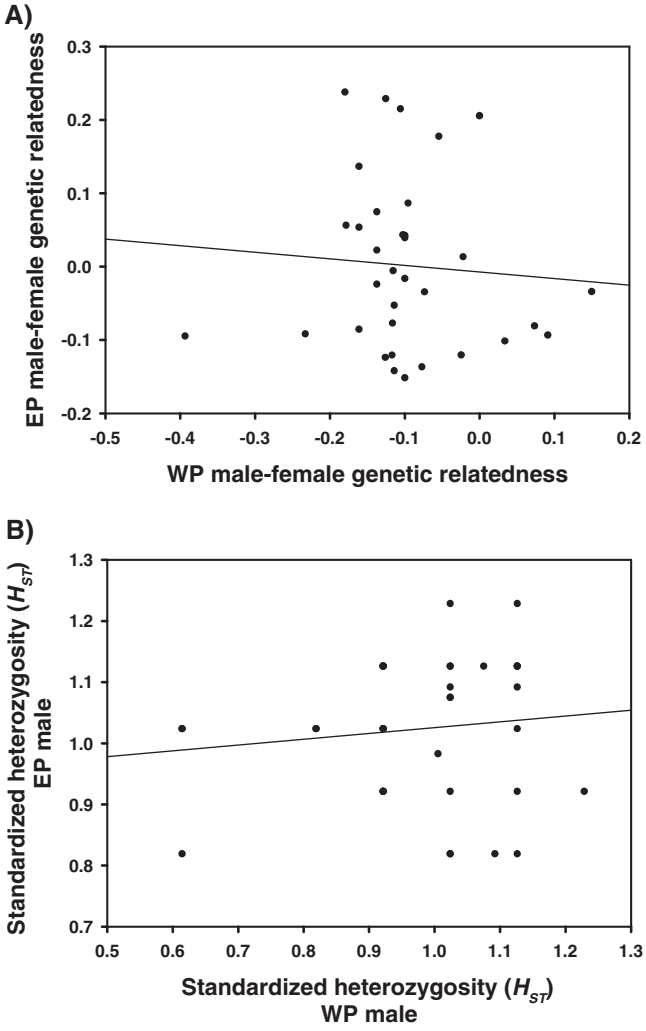

Figure 5
Comparisons between extrapair and within-pair males. (A) Correlation between the genetic relatedness of the social (within-pair) male and the extrapair male to the female. *x* axis represents the genetic relatedness between the within-pair male and the female; the *y* axis represents the genetic relatedness between the extrapair male and the female. WP: within-pair, EP: extra-pair. (B) Correlation between the standardized heterozygosity of the within-pair male and the standardized heterozygosity of the extrapair partner. *x* axis represents standardized heterozygosity of the within-pair male; *y* axis represents standardized heterozygosity of the extrapair male. H_{ST}: standardized heterozygosity, WP: within-pair, EP: extra-pair.

sired multiple offspring at neighboring boxes, suggesting the potential for a compensatory benefit. In the study conducted by Kempenaers et al. (1999) on the Tree Swallow, in which all the breeding males in the population were captured and sampled, the authors found that only 21% of the extrapair young were sired by resident males, suggesting that females obtain most of their extrapair fertilizations outside localized concentrations of nesting sites. White-rumped Swallows in our nestbox population might show a similar mating pattern, with some birds obtaining extrapair copulations outside the breeding colony. However, when we compared the White-rumped Swallow mating pattern to that in the Tree Swallow, we found that a smaller proportion of White-rumped Swallow females procured copulations beyond the limits of our colony, as we were able to assign a higher percentage of extrapair offspring (46.63% vs. 21% in Tree Swallows) to one of the resident

Table 3
Results of the randomization tests for the genetic similarity of the social male to the female and the extrapair male to the female

	Observed mean	Observed SD	*N*	Randomized mean	Randomized SD	*P*
Social mates	−0.0527	0.1183	52	−0.0112	0.1210	0.006
Extrapair mates	0.0014	0.1156	34	−0.0108	0.1205	0.275

SD, standard deviation.

males in the local population—even when only 75% of these males were sampled.

Although female White-rumped Swallows that have EPY in their nests did not lay significantly different numbers of eggs than females that have all WPY, the former fledged more young (Figure 1). Nestlings at our colony most often died due to starvation because of reduced parental provisioning (i.e., not all nestlings die, but the ones that die are usually found in the nest cup), a combination of starvation and hypothermia because of inclement weather (i.e., all nestlings found dead in the nest-box) or predation events (i.e., nest appears empty at a time where nestlings are too young to have been able to fledge). There are 3 possible interpretations for the differential survival of nestlings in nests of different status. First, females that have extrapair young might be better at providing parental care. This would be consistent with the "constrained female hypothesis" proposed by Mulder et al. (1994) and Gowaty (1996) that suggests that females that are better parental care providers, and which can potentially bear the costs of reduced male care, should be the ones to engage in extrapair behavior. A second possibility is that males mated to the females that engage in extrapair behavior are good parental care providers—which might mean that they spend more time defending the nests from intruders and predators and/or they are better than other males at providing food for the nestlings. If males spend more time at their nests, their females might be able to judge through behavioral cues the quality of their social mate as a parent, and even engage in extrapair copulations during their fertile period more freely, given the open opportunity for increased chances for undisturbed copulations. A third alternative is that these differences are driven by some fitness advantage of the extrapair offspring (i.e., some offspring are more resilient to times of hardship). We can divide these alternatives into 2 more inclusive categories: Males and/or females are driving these differences in survival by being better parents, or these differences reflect the quality of the extrapair offspring.

In order to differentiate between these 2 scenarios, we compared the fate of EPY and WPY. Overall, EPY had a higher probability of surviving the nestling period than did WPY. Similarly, nests with all EPY had the highest probability of fledging young and nests with all WPY, the lowest. However, when only nests of mixed paternity were used in this comparison, we failed to find differences in survival between EPY and WPY. That is, within nests, there was no difference in survival among young fathered by different sires—there does not seem to be an intrinsic advantage for the EPY in terms of their survivorship. Thus, the overall differences in fate between EPY and WPY, when all nests were considered, were likely driven by the parents: In nests where there are one or several EPY, the social parents might be better parental care providers than the social pair in nests with all WPY.

Heterozygosity, paternity, and reproductive success

Under the heterozygosity hypothesis (Zeh JA and Zeh DW 1996, 1997; Brown 1997) offspring heterozygosity should be negatively related to the genetic similarity of their biological parents (Prediction 1 Introduction). In addition, EPY should be more heterozygous than WPY (Prediction 2 Introduction), and offspring that are more heterozygous should have higher fitness (Prediction 3 Introduction), after controlling for the shared maternity at the nest. Moreover, females should mate with males having alleles that best complement their own and thus increase offspring heterozygosity—and, as a result, offspring fitness—or they should mate with males that are more heterozygous—and, as a result, are of "better quality." Under this scenario, the genetic similarity between the social pair should predict mating behavior: Females should engage in extrapair behavior more often in those cases in which the pair is genetically similar (Prediction 4 Introduction), and the extrapair male should be less genetically similar to the female than the within-pair male (Prediction 5 Introduction). In addition, the social male's heterozygosity should predict mating behavior (Prediction 6 Introduction) if females will preferentially mate with males whose genetic quality is determined by their level of heterozygosity at one or several loci. In our study, we found support for the first prediction: Offspring that were more heterozygous had sires that were less genetically similar. We found, however, that EPY and WPY did not differ in their level of heterozygosity (Prediction 2 Introduction), and nestlings that died before fledging were more heterozygous than those that fledged (Prediction 3 Introduction). That is, the relationship between heterozygosity and survival of offspring was significant but in the opposite direction as the one expected by the heterozygosity hypothesis. In our analyses, we found that neither male heterozygosity nor the genetic similarity between the nest-attending adults were good predictors of EPP status (Prediction 4 Introduction). However, when we compared the difference in genetic similarity between the female and her social mate with that of the female with her extrapair mate, we did find significant differences, but in a direction opposite to what was expected by theory—females chose social partners with whom they shared a lower degree of genetic similarity, when compared with their extrapair partners (Prediction 5 Introduction). Moreover, the results of the randomization tests showed that females partnered with social males that were less genetically similar than a random choice of males in the population, but nonetheless, the EPP rates in the population were high (77% nests, Table 2). Last, we did not find significant differences in the levels of heterozygosity between extrapair and within-pair partners (Prediction 6 Introduction).

There are 2 points that can be derived from our results. First, given the negative correlation between the offspring's heterozygosity level and the genetic similarity of the parents, how is it possible that EPY and WPY do not differ in heterozygosity, but extrapair males and within-pair males do differ in their degree of genetic similarity with the social female? One explanation may be that females engage in many extrapair copulations with the available males but that fertilization success biases the outcome of such copulations (Griffith and Immler 2009). Zeh JA and Zeh DW (1997, 2008) suggest that the female's reproductive tract provides a physiologically hostile environment where incompatible sperm and embryos are screened (e.g., failed

fertilization, early interruptions in the development of the embryo, etc.). In such cases, selection on heterozygosity will occur at the gamete or embryo level and could result in EPY and WPY having similar levels of heterozygosity (Zeh JA and Zeh DW 2008), even if females prefer to mate with extrapair males that are more similar to themselves (as shown here) or more different (as in other studies) in response to selective pressures in the environment. In birds, sperm are stored in specialized sperm storage tubules located in the female's reproductive tract, and these tubules comprise a common arena in which sperm competition can take place (Birkhead 1988). Thus, sexual conflict paired with sexual selection may result in the pattern observed in which selection occurs postcopulation within this tract or even postfertilization.

Second, the heterozygosity hypothesis for female mate choice remains controversial (reviewed in Akçay and Roughgarden 2007 and Wetzel and Westneat 2009). Although there seems to be growing evidence that selection might drive females to avoid the costs of inbreeding by selecting extrapair males that increase the heterozygosity of their offspring (Zeh JA and Zeh DW 2008; Griffith and Immler 2009), some studies have found no support for the heterozygosity hypothesis (e.g., Kleven and Lifjeld 2005; Smith et al. 2005; Stewart et al. 2006). Our study is different, however, in that we found females to be less genetically related to their social partners than to their extrapair partners and than to a random choice of males in the population and found offspring with higher values of heterozygosity to have a lower survival probability. Thus, our results not only do not find support for the heterozygosity hypothesis, but they contradict its predictions. Although female choice may not be the only explanation for the extrapair behavior observed in this system, under this scenario in which females choose social and extrapair males based on some behavioral, phenotypic, or genetic attribute, their choice of a partner might differ depending on context (e.g., females might choose social males with whom they are more genetically compatible but mate with extrapair partners with whom they have an increased opportunity for mating—neighbors, males they encounter while foraging or at roosting sites).

In many ways, the patterns in White-rumped Swallows appear to be the mirror image of those from a similar study conducted on its congener, the Tree Swallow, in Canada (sample sizes for this study were similar to ours, $N = 502$ offspring and 99 broods typed at 11 microsatellite loci, Stapleton et al. 2007). In that study, the authors found that EPY were more heterozygous than WPY, but social mates and extrapair mates did not differ in their levels of genetic similarity with the female, and the genetic similarity with the social mate did not predict the presence of EPY. In addition, they did not find a difference in heterozygosity between offspring that died and those that fledged. The authors also found that the more heterozygous EPY were a result of copulations outside of the breeding colony. Thus, the situation in Tree Swallows appears to be opposite that in White-rumped Swallows: Female Tree Swallows increased offspring heterozygosity through extrapair matings, despite the lack of difference in the female's genetic similarity between extrapair and within-pair mates, and there did not seem to be an obvious fitness effect on more heterozygous offspring.

CONCLUSIONS

White-rumped Swallows engage in extrapair matings that result in a high proportion of EPY, at least half of which are sired by males within the colony. Despite the seeming failure of heterozygozity to explain extrapair mating in this species, there remain interesting patterns to be interpreted. Although we did not find fitness benefits associated with extrapair behavior, the relationship between reduced heterozygosity and increased fitness of offspring, as well as the reduced genetic similarity between the social pair, are topics that will benefit from further study and might provide a better insight to the study of mating systems and sexual selection. We found a fitness advantage for nests that had EPY—as more young fledged from these nests—but this advantage seems most likely due to the adults at those nests being better at providing parental care or interacting in some way differently from those that had only WPY. Consequently, it appears that in White-rumped Swallows, heterozygosity increase is not the selective outcome of extrapair female mate choice, a reversal of the pattern previously found in Tree Swallows. Are mixed and extrapair brood females better females that are able to compensate for any potential retaliation from their cuckolded mates (Gowaty 1996), or are cuckolded males for some reason more willing or able to contribute parental care? These questions remind us that different species under different ecological conditions and with similar evolutionary heritages might show completely different modes of mate choice and parental interactions.

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