

Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine

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Abstract Extra-pair paternity is common in socially monogamous passerines; however, despite considerable research attention, consistent differences in fitness between within-pair offspring (WPO) and extra-pair offspring (EPO) have not been demonstrated. Recent evidence indicates that differences between maternal half-siblings may depend on environmental conditions, but it is unclear whether the influence of paternal genetic contribution should be most apparent under comparatively poor or favourable conditions. We compared phenotypic characteristics of WPO and EPO in 30 mixed-paternity broods of the tree swallow (*Tachycineta bicolor*) in relation to experimentally increased nest temperature ($n=13$ heated nests; 17 control nests) and natural abundance of haematophagous parasites (*Protocalliphora* spp.). This allowed us to test the hypothesis that genetic benefits of extra-pair mating are environment dependent. EPO grew their ninth primary feathers faster than WPO regardless of nest temperature or parasite load and had significantly longer ninth primary feathers at fledging when parasite abundance was low, and when they were positioned early in the hatching sequence relative to WPO. In contrast, WPO under similar conditions did not differ from EPO in any phenotypic trait measured. These results indicate that the fitness benefits of extra-pair mating are likely to be context dependent, and that genetic effects on some phenotypic traits may be more apparent when conditions are relatively favourable.

Keywords Extra-pair paternity · Good genes · Context dependence · Fitness consequences · *Tachycineta bicolor*

Introduction

Extra-pair paternity is common in socially monogamous passerines (Petrie and Kempenaers 1998), and because extra-pair males generally do not provide material benefits, such as territory access or parental care, it is likely that females seek extra-pair fertilizations to obtain indirect benefits, such as high-quality genes for their offspring (Griffith et al. 2002). Despite considerable research attention, however, consistent differences in fitness between within-pair offspring (WPO) and extra-pair offspring (EPO) have not been demonstrated (Griffith et al. 2002). In some species, EPO are more likely to survive (Kempenaers et al. 1997), fledge in a better condition (Sheldon et al. 1997) or exhibit enhanced immune responses (Johnson et al. 2000) relative to their within-pair nest mates. In other species, however, no such differences have been found (e.g. Lubjuhn et al. 1999; Whittingham and Dunn 2001; Kleven and Lifjeld 2004). Consequently, the extent to which female birds select extra-pair mates to obtain indirect genetic benefits for their offspring is not known.

Genetic benefits of extra-pair mate choice should be evident in comparisons of phenotypic characteristics of WPO and EPO, as many traits are both heritable (Ryan 2001) and strongly correlated with future reproductive success or survival in birds (Gebhardt-Henrich and Richner 1998; McCarty 2001). The expression of genetic variation in phenotypic traits, however, commonly varies with environmental conditions (e.g. Merilä 1997; Qvarnström 1999). Thus, the magnitude of phenotypic differences between WPO and EPO that are due to paternal genetic

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contribution may depend on the conditions of the rearing environment. Context dependence of genetic benefits of mate choice has been demonstrated in a range of taxa, including invertebrates, amphibians and birds (Jia et al. 2000; Welch 2003; Schmoll et al. 2005). To our knowledge, however, only one study has considered context dependence in comparisons of WPO and EPO of socially monogamous birds (Schmoll et al. 2005), and it is unclear whether context-dependent genetic benefits of mate choice should be more evident in low or high stress environments (e.g. Welch 2003; Schmoll et al. 2005). Hence, consideration of genotype-environment interactions may be necessary to identify genetic benefits of extra-pair mating in socially monogamous birds and to determine the range of environmental conditions, under which selection for extra-pair mate choice by females may be maintained in natural populations.

We compared phenotypic characteristics of maternal half-siblings in 30 mixed-paternity broods of tree swallows (*Tachycineta bicolor*) exposed to varying environmental conditions. We tested the effects of experimentally increased nest temperature, as well as the naturally occurring abundance of *Protocalliphora* spp., on relative performance of WPO and EPO, as measured by growth and size at fledging. Experimentally heating nests has been shown to enhance growth and size at fledging for mass and ninth primary feathers in tree swallows (Dawson et al. 2005a). This enhanced growth of chicks in heated nests appears to be due to a reduction in thermoregulation costs, allowing greater allocation of resources to growth compared to chicks in unheated nests (Dawson et al. 2005a). In addition, nest-dwelling ectoparasitic haematophagous blow fly (*Protocalliphora* spp.) larvae have significant negative impacts on nestling survival and condition at fledging in a range of bird species, including cavity nesters (e.g. Merino and Potti 1996; O'Brien et al. 2001). If female tree swallows engage in extra-pair mating to obtain genetic benefits for their offspring and these benefits are context-dependent, we predict that the performance of EPO relative to their within-pair half-siblings will depend on the level of thermal stress or parasite infestation to which nestlings are exposed.

Materials and methods

Study area and study species

We studied tree swallows breeding in nest boxes near Prince George, BC, Canada (53°N, 123°W) from May to August 2004. The site contained 154 nest boxes mounted on fence posts and placed approximately 25 m apart. The study area consists of open agricultural fields mixed with patches of coniferous and deciduous forest, as well as many

small wetlands, and is located in a region characterized by a temperate climate, with cool summer temperatures.

Tree swallows are small, migratory, socially monogamous passerines (Robertson et al. 1992) that exhibit among the highest rates of extra-pair paternity identified in birds: extra-pair young have been detected in up to 90% of broods, with as many as 69% of all offspring resulting from extra-pair fertilizations (Barber et al. 1996; Conrad et al. 2001; Whittingham and Dunn 2001). Females do not gain direct benefits from extra-pair mates, yet no consistent indirect benefits of extra-pair mating, in terms of offspring quality or survival, have been identified in this species (Whittingham and Dunn 2001; Whittingham et al. 2003).

Field and experimental methods

Beginning in May, we visited nests every 2 days to determine clutch initiation date and clutch size. Near expected hatch dates, nests were visited every day to determine date of hatch. Adults were captured in nest boxes with a swing-door trap. We banded adults and 14-day-old nestlings with standard aluminium leg bands and collected blood samples (approximately 50 µl) by puncturing the brachial vein.

Nests were paired by hatching date and brood size, and one nest from each pair was randomly assigned to either the heating treatment or control group. Using methods described in Dawson et al. (2005a,b), we experimentally increased temperature of treatment nests ($n=15$) by installing small heating pads in nests when young were 4 days old until they were 16 days of age. These pads, which were changed every 2 days, produced heat when the iron powder they contained was exposed to air and oxidized. Control nests ($n=18$) contained pads in which the iron powder had been fully oxidized, so heat was not produced. Although we did not measure nest temperatures in this study, this heating treatment has been shown to increase nest temperature by up to 10°C relative to control nests (Dawson et al. 2005a). Treatment and control nests, therefore, represented low and high thermal stress conditions, respectively. Once chicks had fledged, nesting material was collected from each box and stored in sealed bags at room temperature for 14 days. We then sifted through the nesting material to remove all pupae of *Protocalliphora*. This procedure has been used to quantify parasite abundance in previous studies of blow flies (e.g. Hurtrez-Boussès et al. 1997; Dawson et al. 2005b), with the number of pupae representing the minimum level of infestation. Because the abundance of *Protocalliphora* increased with brood size (see “Results”), we used the number of pupae per nestling as a relative measure of parasite load in each nest.

Parents may have responded to the nest temperature manipulation or the relative abundance of nest-dwelling

ectoparasites by adjusting provisioning rates (Tripet and Richner 1997), which could directly influence nestling performance. We measured parental provisioning rates by placing a digital recorder and small microphone in nest boxes to record begging of chicks. Recordings of at least 1 h (1.46 ± 0.08 h SE, $n=55$ recordings from 33 nests) were obtained when chicks were 12–14 days old; most nests were recorded on more than 1 day within this interval, which allowed us to calculate an average provisioning rate for each nest. The number of bouts of begging accurately represents the number of parental visits to nest boxes (E. L. O'Brien, unpublished data), and because parent tree swallows rarely visit nest boxes without feeding chicks (McCarty 2002; Whittingham et al. 2003), the frequency of parental visits is an accurate measure of the rate of food delivery to nestlings (McCarty 2002).

We measured nestling mass, tarsus and ninth primary feather length every 2 days from ages 4 to 16 days. When chicks were 9 days old, we tested the T cell-mediated immune response (CMI) by injecting 30 μ l of 1 mg/ml phytohaemagglutinin-P (Sigma) in phosphate buffered saline intradermally in the right wing web. For each nestling, thickness of the wing web was measured four times using a thickness gauge (nearest 0.01 mm) immediately before and 24.07 h (± 0.01 SE, $n=172$) after injection; the means of these four measurements were then used in all subsequent analyses. We determined CMI as the difference in thickness of the wing web before and after injection (Smits et al. 1999). Growth rate constants for morphological traits were calculated using the logistic model for growth of mass, Gompertz model for growth of tarsus and a linear model for growth of ninth primary. Model selection procedures followed Dawson et al. (2005a).

Parentage analysis

Blood samples were stored in 1 ml of Queen's lysis buffer (Seutin et al. 1991) at 4°C. We isolated genomic DNA by proteinase K-phenol/chloroform extraction, and DNA was subsequently resuspended in Tris-EDTA (TE) buffer and stored at -20°C. We used the following three microsatellite loci for paternity analysis: HrU6 (Primmer et al. 1995), HrU10 (Primmer et al. 1996) and IBI MP5-29 (Crossman 1996). Polymerase chain reaction (PCR) amplification was carried out in 10- μ l volumes containing 50–100 ng genomic DNA, 100 μ M deoxyribonucleotide triphosphates, 2.5–3.0 mM MgCl₂, 1 \times PCR buffer (Invitrogen), 0.2 μ M forward (labelled) and reverse primers and 0.35 units of Taq DNA polymerase (Invitrogen). PCR reactions were performed using an MJ Research Peltier thermal cycler under the following conditions: for HrU6, 1 cycle at 94°C for 2 min, followed by 40 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 1 min and one final extension step at

72°C for 4 min. Conditions for HrU10 and IBI MP5-29 were identical except we used annealing temperatures of 59°C and 57°C, respectively. PCR products were analysed using a Beckman-Coulter CEQ 8000 automated sequencer.

Two of the three loci were highly polymorphic (Table 1), and the exclusion probability for all three loci (Jamieson 1994) was 0.995 with one parent known. All nestling genotypes were compatible with maternal genotypes at IBI MP5-29, but mismatches at either HrU6 or HrU10 occurred in 52/216 (24%) of nestlings. In all of these cases, either the female or the nestling was apparently homozygous at the mismatching locus, indicating the presence of null alleles at both HrU6 and HrU10 in this population. This was confirmed by null allele frequency estimates generated by Cervus 2.0 (Marshall et al. 1998), based on genotypes of 111 adults captured in 2004 (Table 1). We classified nestlings as EPO if they did not share an allele with their social father at minimally two of the three loci. To account for the presence of null alleles, however, we did not include mismatches involving nestlings who were apparent homozygotes at either HrU6 or HrU10, unless their allele also mismatched the attending female (indicating the maternal allele failed to amplify), and the social father was heterozygous at that locus. Out of all EPO we identified, 53/76 (70%) mismatched their social father at two loci, and 23/76 (30%) mismatched at all three loci. Nestlings that shared an allele with their social father at two (41/141; 29%) or three (100/141; 71%) loci were considered WPO.

Statistical analysis

We used paired *t*-tests to compare mean values of phenotypic traits for EPO and WPO in each nest containing offspring of mixed paternity (30 nests; see “Results”). This allowed us to initially determine whether EPO differed from their maternal half-siblings across all nests. To assess whether environmental conditions influenced relative performance of half-siblings, we used analysis of covariance to test for effects of the heating treatment as a fixed factor, and *Protocalliphora* abundance (number of pupae/nestling),

Table 1 Variability of microsatellite loci for 111 adult tree swallows

Locus	Number	No. alleles	P_{ei}	h_e	h_o	Null frequency
HrU6	108	55	0.839	0.919	0.833	0.046
HrU10	111	69	0.956	0.983	0.811	0.094
IBI MP5-29	111	6	0.337	0.612	0.649	0.000

P_{ei} is the probability of exclusion with one parent (female) known, h_e is the expected heterozygosity and h_o is the observed heterozygosity. The combined probability of paternal exclusion was 0.995.

provisioning rate (feeds/hour/nestling), brood size and hatching date as covariates, on the relative performance of EPO and WPO. Position in the hatching sequence affects relative nestling performance (e.g. growth or size at fledging) in many altricial birds, including tree swallows (Clotfelter et al. 2000), and both hatch order and asynchrony can be estimated by relative body mass soon after hatch (Clotfelter et al. 2000). To account for within-brood phenotypic variation due to position in the hatching sequence, we therefore included difference in mass at day 4 (average mass of EPO – average mass of WPO) as an additional covariate representing relative hatch order. Thus, values >0 indicate that, on average, EPO hatched earlier than WPO, whereas values <0 indicate that WPO hatched earlier than EPO. Similarly, for each phenotypic trait, we used the difference between EPO and WPO (average EPO value–average WPO value) as the dependent variable; thus, values >0 indicate EPO were larger or grew faster than WPO, whereas values <0 indicate the opposite pattern. Initial models tested all main effects and interactions. Terms that were not significant ($P>0.10$) were then removed by backwards stepwise elimination until only significant terms remained, or a significant interaction was detected. We used SPSS (Norušis 2000) software for all analyses. Means are presented ± 1 standard error (SE). All tests were two tailed, and results were considered significant at $\alpha<0.05$ level.

Results

Incidence of extra-pair paternity was similar to other populations of tree swallows. Of 40 nests included in parentage analysis, 34 (85%) contained offspring of mixed paternity. The average proportion of EPO in these nests was 0.43 ± 0.04 . In total, 76/216 (35%) of all offspring genotyped were EPO. Fledging success across all nests in the study population was relatively high: 49/64 (77%) nests fledged at least one young, and 232/347 (67%) chicks survived to fledging. However, EPO were not more likely to survive than WPO (65/76, 86% EPO vs 114/141, 81% WPO; likelihood-ratio test, $G=0.77$, $df=1$, $P=0.38$), nor were they more likely to hatch first (EPO heavier at day 4=17 nests vs WPO heavier=14 nests; $G=0.29$, $df=1$, $P=0.59$). Of the 33 nests included in the heating treatment, three contained only WPO (two heated, one control) and could therefore not be used in comparisons of maternal half-siblings. The remaining 30 nests contained offspring of mixed paternity (13 heated, 17 control). In these nests, extra-pair young grew their ninth primary feathers faster than their within-pair half-siblings (Table 2). EPO also exhibited faster growth rates of mass, and had longer ninth primary feathers at fledging relative to WPO, although these differences were not

Table 2 Results of two-tailed paired *t*-tests comparing phenotypic traits of extra-pair and within-pair offspring in mixed-paternity broods of tree swallows

Trait	Mean difference (\pm SE)	<i>t</i>	df	<i>P</i>
Ninth primary growth (mm/day)	0.12 \pm 0.05	2.21	28	0.04
Mass growth (g/day)	0.02 \pm 0.01	1.94	28	0.06
Tarsus growth (mm/day)	0.01 \pm 0.02	0.51	28	0.61
Ninth primary at day 16 (mm)	0.88 \pm 0.49	1.80	28	0.08
Mass at day 16 (g)	0.27 \pm 0.24	1.14	28	0.26
Tarsus at day 16 (mm)	0.02 \pm 0.06	0.36	28	0.72
T cell-mediated immune response (mm)	0.02 \pm 0.05	0.32	24	0.75

Differences were calculated as mean value for extra-pair offspring (EPO)—mean value for within-pair offspring (WPO) within nests; thus, differences >0 indicate values for EPO were larger, whereas differences <0 indicate values for WPO were larger. These comparisons do not account for variation in environmental conditions and, thus, are for both control and heat treatment groups combined

statistically significant. EPO did not consistently differ from WPO in any other phenotypic measures (Table 2).

The heating treatment did not influence provisioning rates (heated nests: 5.7 ± 0.7 feeds/chick, control nests: 4.7 ± 0.3 ; $t=1.44$, $df=26$, $P=0.16$), nor did heated nests differ from controls in parasite abundance (heated: 6.7 ± 1.1 *Protocalliphora*/nestling, controls: 9.1 ± 1.4 ; $t=1.25$, $df=26$, $P=0.22$). All experimental nests (heated and controls) that contained offspring of mixed paternity were infested with *Protocalliphora* (excluding one nest, for which parasite abundance was not determined). The average number of *Protocalliphora* detected was 45.6 ± 32.2 (range, 3–122; $n=29$). This level of infestation is similar to that reported in nests of tree swallows in other study populations (e.g. Rogers et al. 1991; Dawson et al. 2005b). Nests with larger broods contained more *Protocalliphora* pupae ($r=0.48$, $P=0.009$, $n=29$), so all subsequent analyses used *Protocalliphora*/nestling as a covariate representing level of parasite infestation.

The heating treatment did not affect relative nestling performance for any phenotypic trait measured (all $P>0.10$), and we suspect this was because weather conditions in the study area during 2004 were relatively benign (unpublished data). In the analysis of difference in length of ninth primary at day 16, however, we detected a significant interaction between relative hatch order and level of parasite infestation ($F_{1,24}=12.79$, $P=0.002$). When we considered only nests in which EPO hatched first (difference in mass at day 4>0), *Protocalliphora* abundance was a significant predictor of the relative length of ninth primary feathers ($F_{1,14}=7.37$, $P=0.02$; Fig. 1). Indeed, under low to moderate levels of infestation

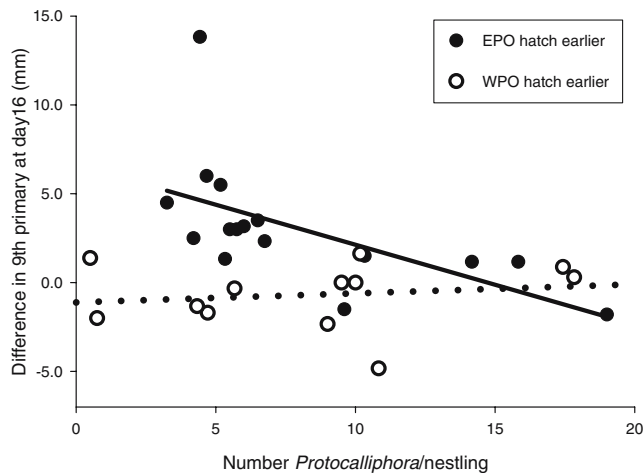


Fig. 1 Influence of parasite (*Protocalliphora* spp.) abundance and position of nestlings in the hatching sequence on the difference in length of ninth primary at day 16 between extra-pair offspring (*EPO*) and within-pair offspring (*WPO*) in 29 nests of tree swallows. Differences were calculated as mean value for *EPO* – mean value for *WPO* within nests; thus, differences >0 indicate *EPO* had longer primaries at day 16, while differences <0 indicate primaries of *WPO* were longer. The *solid line* represents the regression line for nests in which *EPO* hatched earlier (difference in length of ninth primary at day 16 = $6.64 - 0.59(\text{number } \textit{Protocalliphora}/\text{nestling})$; $R^2=0.35$), while the *dotted line* is the regression line for nests in which *WPO* hatched earlier [difference in length of ninth primary at day 16 = $-1.12 + 0.05(\text{number } \textit{Protocalliphora}/\text{nestling})$; $R^2=0.02$]. The relationship between parasite load and difference in length of ninth primary for nests in which *EPO* hatched earlier remained significant after removal of the outlier

(fewer than the mean of 8.0 *Protocalliphora* per nestling), *EPO* grew their primaries longer relative to *WPO* (average difference = 4.61 ± 1.02 mm; paired *t*-test: $t=4.52$, $df=10$, $P=0.001$). There was no additional effect of degree of hatching asynchrony (i.e. the magnitude of the difference in mass at day 4) between *EPO* and *WPO* on relative length of ninth primary in these nests ($F_{1,13}=2.76$, $P=0.12$). In contrast, in nests where *WPO* hatched earlier (difference in mass at day 4 <0), neither *Protocalliphora* abundance ($F_{1,8}=0.54$, $P=0.48$; Fig. 1) nor degree of hatching asynchrony ($F_{1,8}=2.00$, $P=0.20$) affected relative ninth primary length. Differences between *EPO* and *WPO* in mass and tarsus at day 16, as well as relative CMI response, were not influenced by *Protocalliphora* abundance, provisioning rate or relative hatch order (all $P>0.14$). Difference in tarsus growth rate was related only to hatch order: nestlings positioned early in the hatching sequence (i.e. heavier at day 4) grew their tarsi faster ($F_{1,28}=11.88$, $P=0.002$), irrespective of differences in paternal genetic contribution. Provisioning rates (feeds/hour/nestling) were higher in more heavily infested nests across the entire study population ($r=0.35$, $P=0.03$, $n=39$), however, provisioning rate was not a significant predictor of relative nestling performance in any of the above analyses (all $P>0.28$).

Discussion

Expression of genetic variation in phenotypic traits often varies with environmental conditions (Qvarnström 2001), and genetic benefits of extra-pair mate choice in socially monogamous birds may therefore not be evident in all environments. Our results support this hypothesis, as *EPO* positioned early in the hatching sequence produced longer flight feathers than their within-pair half-siblings in nests that contained few parasitic blow fly larvae, whereas *WPO* that hatched early did not show a similar advantage under low parasite loads (Fig. 1). Wing length is a strong predictor of timing of fledging in tree swallows (Michaud and Leonard 2000) and other aerial insectivores (Martins 1997); thus, in some rearing environments, *EPO* may reach fledging condition earlier than *WPO*. Fitness benefits of longer flight feathers may also extend beyond the fledging period in swallows. For example, wing length at fledging is positively associated with probability of recruitment (McCarty 2001), and males with longer flight feathers attract social mates of higher quality and are more likely to sire extra-pair young (E. L. O'Brien, unpublished data). Finally, females who exhibit superior flight performance, a behaviour influenced by traits such as wing length, lay more eggs and initiate clutches earlier in the breeding season (Bowlin and Winkler 2004). For female tree swallows, the fitness benefits of producing *EPO* in some contexts (e.g. when parasite abundance is low) may therefore be substantial, and the context dependence of these benefits may explain why previous studies have failed to find significant effects (e.g. Whittingham and Dunn 2001).

For traits such as body size or growth, phenotypic expression of genetic variation may be enhanced in favourable environments, where nutrient limitation is minimal (Hoffman and Merilä 1999). Studies of heritability (Qvarnström 1999) and mate choice (Welch 2003) have supported this prediction, and the results of our study suggest that some genetic benefits of extra-pair mating in socially monogamous birds may similarly be realized under low stress conditions (i.e. low parasite load). However, the alternative hypothesis that stressful conditions should promote the expression of heritable phenotypic variation (Hoffman and Merilä 1999) has also received empirical support (e.g. Sheldon et al. 2003; Schmoll et al. 2005), and this discrepancy suggests that selection on phenotypic variation may not be consistently strongest under either high- or low-stress conditions. Whether unfavourable environments constrain or enhance expression of heritable variation in phenotypic traits may depend on the stressor and traits considered (see Malausa et al. 2005). For example, high levels of parasitism may enhance expression of genes associated with immune resistance while reducing expression of growth-promoting loci; under these condi-

tions, variation in traits such as immune response should be more apparent and differences in body size or growth reduced (e.g. Bize et al. 2003). For tree swallows, neither parental provisioning rates nor manipulation of nest microclimate affected relative performance of maternal half-siblings (in terms of growth or size at fledging), whereas phenotypic differences between EPO and WPO were evident when parasite abundance was low. Because we did not record nest temperatures in this study, we cannot exclude the possibility that the heating treatment did not have the desired effect on microclimate of nests. However, this method has previously been shown to increase temperatures in nests of tree swallows in the same geographic area (Dawson et al. 2005a), and we believe it is more likely that unusually mild weather during the year of this study (unpublished data) resulted in all nestlings experiencing relatively low thermal stress. It is therefore possible that abiotic factors, such as nest microclimate, can influence the relative performance of half-siblings in some environments; however, our results suggest that stresses due to environmental factors, such as feeding conditions or nest temperature, may have less potential to influence within-brood variation in phenotypic traits of nestlings in this species than factors such as parasites. Future studies investigating the fitness benefits of extra-pair paternity in socially monogamous birds should therefore examine phenotypic responses of offspring exposed to a wide range of conditions, representing both poor and favourable environments.

Preferential allocation of limited resources to growth of morphological traits that have high functional priority occurs in a range of taxa, including mammals (e.g. Festa-Bianchet et al. 2004) and birds (Ashton and Armstrong 2002; Bize et al. 2003). In aerial insectivores, allocation to growth of wing feathers may be particularly important for fledging (Martins 1997) and adult survival (Brown and Brown 1998). Differences in the tendency to preferentially allocate resources to wing growth may therefore represent an important source of variation in nestling quality. Across all environmental conditions and regardless of relative hatching order, EPO of tree swallows grew wing feathers faster than their WPO half-sibs (Table 2) and, under favourable conditions, had considerably longer ninth primaries at fledging (Fig. 1). This suggests that EPO had a greater tendency to preferentially allocate resources to wing growth than WPO in our study population. More generally, these results highlight the potential importance of examining heritability of resource allocation strategies, and how these strategies influence phenotypic traits under varying levels of resource availability.

Female birds may adjust yolk androgen investment (Gil et al. 1999), egg or clutch size (Cunningham and Russell 2000; Parker 2003) according to the attractiveness of their mates. Thus, phenotypic differences between WPO and

EPO may reflect differential allocation of resources among offspring of different sires in addition to effects of paternal genetic contribution (Sheldon 2000). We are unaware of any evidence, however, that female birds are capable of adjusting relative investment to specific eggs within a clutch (see Birkhead et al. 2000) except in relation to laying order (e.g. Saino et al. 2001). Because EPO of tree swallows are not consistently positioned early or late in the hatching sequence (this study; see also Whittingham et al. 2003), differential allocation of maternal resources based on laying order would not allow females to preferentially invest in EPO in this species. Similarly, although male nestlings of tree swallows are larger than their female siblings by day 12 (Whittingham et al. 2003), EPO are not more likely to be male (Whittingham and Dunn 2001), and phenotypic differences between WPO and EPO can therefore not be attributed to differences in sex allocation according to paternity. Phenotypic differences between maternal half-siblings are also unlikely to have resulted from biased distribution of parental feedings: allocation of provisioning effort among nestlings by both parents is generally equitable in tree swallows (Leonard and Horn 1996) regardless of the presence or abundance of EPO in the brood (Whittingham et al. 2003). It is therefore unlikely that phenotypic differences between WPO and EPO in this study can be attributed to differential allocation of resources to EPO.

Expression of heritable variation in phenotypic traits varies with environmental conditions (e.g. Merilä 1997; Qvarnström 1999). Hence, the influence of paternal genetic contribution on performance of WPO and EPO should not necessarily be evident in all contexts. Results of this study support the hypothesis that genetic benefits of extra-pair mating by female tree swallows are environment dependent and, contrary to the findings of Schmoll et al. (2005), these benefits were realized under comparatively favourable conditions (low parasite abundance). Clearly, future tests of ‘good genes’ models of female extra-pair mate choice should consider environmental variation in comparisons of maternal half-sibling performance not only to identify genetic benefits of extra-pair paternity but also to determine the range of environmental conditions under which selection for extra-pair mate choice by females is maintained.

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