Social and Extra-pair Mate Choice by Female Tree Swallows: The Importance of Male and Nest-site Quality, and Consequences for Offspring Performance

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Abstract

Extra-pair paternity is common among socially monogamous birds, yet our understanding of the factors influencing female selection of social and extra-pair mates, as well as the fitness consequences of extra-pair fertilizations, remains limited for many species. Using a combination of nest-site manipulations and paternity analyses, I studied tree swallows (*Tachycineta bicolor*) breeding in central British Columbia to examine (1) the relative importance of nest-site and male quality for selection of social mates by females, (2) phenotypic characteristics associated with extra-pair mating success in resident males, and (3) the consequences of extra-pair paternity for offspring performance.

In one of the two study populations, birds were less likely to occupy nest boxes that had a high perceived risk of ectoparasitism, suggesting that choice of social mates in this population is influenced by nest-site quality. In both populations, however, female tree swallows produced smaller clutches in high-risk (treatment) nest boxes. This difference was not explained by phenotypic characteristics of females or their social mates, indicating that nest-site quality, though not consistently influencing settlement decisions, may have important effects on primary reproductive investment of female tree swallows. In contrast, male quality was associated with extra-pair mating success: males who sired extra-pair offspring had longer flight feathers, were more likely to be returning breeders, attracted social mates who tended to breed earlier, and raised nestlings with higher growth rates of ninth primary feathers compared to males producing only within-pair offspring. These results support the hypothesis that extra-pair mating allows female tree swallows to obtain good genes for their offspring. Additional studies are needed to examine the extent to which flight feather length varies independent of male age in tree swallows, and thus, whether this trait may be influenced by sexual selection. Finally, comparisons of maternal half-siblings showed that extra-pair offspring grew their ninth primary feathers faster under all conditions, but differences in length of ninth primaries at fledging were only evident under low-stress conditions, when extra-pair offspring were positioned early in the hatching sequence and the intensity of blow fly (*Protocalliphora* spp.) parasitism was low. Within-pair offspring exposed to favourable conditions did not show a similar advantage. This indicates that genetic benefits of extra-pair paternity depend on the environmental conditions to which nestlings are exposed, and extra-pair mating may therefore represent a 'bet-hedging' strategy in response to environmental unpredictability. Consequently, I suggest that tests for genetic benefits of extra-pair fertilizations in socially monogamous species should consider the potential influence of environmental conditions on the phenotypic expression of genetic variation in offspring.

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1. General Introduction

Female mate choice is an important selective force influencing the evolution of male phenotypic characteristics (Møller and Birkhead 1994). Selection may be imposed directly through female preference for high-quality males, or indirectly through female preference for high-quality territories, where male competition for territory access results in a positive correlation between male and territory quality (Sætre et al. 1994). However, it is also possible for females of socially monogamous species to access a high-quality territory through the choice of their social mate, while maximizing the genetic quality of offspring through extra-pair copulations with other higher-quality males (Alatalo et al. 1986). This may be a common strategy, as molecular analyses have revealed high rates of extra-pair paternity in many socially monogamous birds (Petrie and Kempenaers 1998).

Extra-pair mating can be costly for females (e.g. reduced parental investment by social mates, increased risk of predation, disease or parasite infestation; Ligon 1999), and since most do not obtain direct benefits from extra-pair mates (e.g. parental care), it is predicted that extra-pair mating allows females to obtain indirect genetic benefits for their offspring (Jennions and Petrie 2000). These benefits may include good genes or compatible genes (Neff and Pitcher 2005); although these alternatives have commonly been assessed separately, female mating preferences may also lead to optimal combinations of both benefits (Mays and Hill 2004; Neff and Pitcher 2005). Indeed, a number of studies have shown that extra-pair offspring are of higher phenotypic quality (e.g. Johnsen et al. 2000), more likely to fledge (Foerster et al. 2003) or to recruit as breeding adults (Schmoll et al. 2005) relative to their within-pair maternal half-siblings, indicating that the fitness benefits of extra-pair mate choice for females may be substantial (but see Arnqvist and Kirkpatrick 2005).

Extra-pair paternity rates vary considerably among passerines (Griffith et al. 2002); if females assess potential extra-pair mates according to phenotypic traits that serve as reliable indicators of quality, and these traits are heritable, the opportunity for sexual selection on male phenotypic characteristics due to extra-pair fertilizations may be greatest in those species exhibiting the highest rates of extra-pair paternity. Under these conditions, it may therefore be particularly important to identify phenotypic characteristics that predict extra-pair fertilization success of males. Furthermore, such species provide a valuable opportunity to examine phenotypic differences between maternal half-siblings, since a relatively high proportion of nests in any breeding population will contain nestlings of mixed paternity. Thus, the objective of this research was to examine the factors influencing female selection of social versus extra-pair mates, as well as the consequences of mate choice for offspring fitness, in a socially monogamous species known to exhibit high rates of extra-pair paternity, but for which the causes and consequences of extra-pair mating by females are poorly understood.

1.1 Study species and study area

Tree swallows (*Tachycineta bicolor*) are small, aerial insectivorous passerines that breed throughout much of North America. As a secondary cavity nester, this species is limited by availability and quality of nest-sites (Holroyd 1975), which have historically consisted of tree cavities located along the margins of open fields and wetlands (Robertson et al. 1992). Tree swallows readily use artificial nest boxes, however, and have therefore been the subject of considerable research in avian reproductive biology. This species is predominantly socially monogamous, yet exhibits among the highest rates of extra-pair paternity identified in passerines: 50-89% of females produce at least one extra-pair offspring, and between 38-69% of nestlings are sired by extra-pair males (Lifjeld et al. 1993; Barber et al. 1996; Whittingham and Dunn 2001). Females may be capable of raising young on their own; however, parental care by both the male and female is generally required to successfully fledge young (Robertson et al. 1992). Variance in male reproductive success due to extra-pair fertilizations is estimated to be high (Kempenaers et al. 2001; Whittingham and Dunn 2005), yet consistent phenotypic characteristics associated with extra-pair mating success of males have not been identified. Furthermore, it is not known if extra-pair mating has fitness implications for females, in terms of viability or quality of offspring.

The two populations of tree swallows studied for this thesis were located near Prince George BC, Canada (53°N, 123°W), and have been intensively studied since 2001. Each site consisted of approximately 150 nest boxes mounted on fence posts in open fields with patches of mixed forest. The study areas are surrounded by several small wetlands and lakes that provide foraging areas for swallows.

1.2 General objectives

1.2.1 Selection of social mates: nest-site versus male quality

Where attractive male characteristics are correlated with territory quality, female preferences for either male traits or territory characteristics can be difficult to distinguish (Sætre et al. 1994). By controlling for territory quality, some studies have demonstrated female preference for male phenotypic traits (Saetre et al. 1994; Slagsvold and Drevon 1999). In species for which territory or nest-site quality has a significant effect on reproductive success, however, female choice of a social mate may be predicted to be more strongly influenced by territory quality than by male quality. This has been demonstrated in, for example, the side-blotched lizard (*Uta stansburiana*); when male and territory quality were experimentally uncoupled in such a way that lower quality males held better territories, female lizards preferentially selected the best territories, irrespective of male quality. Indirect genetic benefits for offspring were then secured through copulations with high-quality males (Calsbeek and Sinervo 2002).

Secondary cavity-nesting birds such as tree swallows are limited by the availability and quality of nest-sites (Holroyd 1975; Rendell and Robertson 1989; Robertson et al. 1992). Males typically arrive on the breeding grounds up to one week before females, where they immediately establish a territory around a nest-site (Robertson et al. 1992). Female selection of a nest-site involves repeated entry and examination of the cavity (Robertson et al. 1992). It is not known whether the quality of the male occupying the nest-site also influences female choice. In contrast to similar studies in other bird species (e.g. MacDougall-Shackleton et al. 1996), however, male removal experiments in tree swallows demonstrated no change in extra-pair paternity rates following social mate replacement, even though replacement males were smaller (and presumably younger) than the original social mates (Barber et al. 1998). This suggests that female selection of a social mate is not strongly influenced by male quality and, further, that social mates selected by female tree swallows are not superior in genetic quality to surrounding unpaired males. However, the relative importance of male quality versus nest-site quality for female social mate choice in tree swallows has not yet been determined. In chapter 2, I present the results of an experiment conducted over two breeding seasons in which I manipulated the perceived quality of nest-sites prior to female settlement. This allowed me to examine the hypothesis that female tree swallows rely on nest-site quality rather than quality of the resident male when selecting a social mate.

1.2.2 Phenotypic characteristics of extra-pair males

In addition to direct benefits of mate choice, such as access to resources, females may also select mates to obtain indirect benefits such as good genes for their offspring (Hamilton and Zuk 1982). In socially monogamous species, however, female mate choice may be restricted by intrasexual competition (Dale et al. 1992), and energetic costs of searching for a mate (Slagsvold et al. 1988). Although it may be possible for a female to abandon a male that she has already settled with in favour of another male of higher quality (e.g. Dale and Slagsvold 1996; Otter and Ratcliffe 1996), this strategy is contingent on the pairing status of the higher-quality male. Alternatively, females can obtain genetic benefits for offspring through extra-pair copulations with high-quality males, even when those males are already paired. Extra-pair offspring are common in broods of socially monogamous passerines (Petrie and Kempenaers 1998), suggesting this mating strategy may be widespread among female birds.

An important assumption of the good genes hypothesis is that females are able to assess potential mates according to phenotypic traits that serve as reliable indicators of genetic quality (Searcy 1982). This association between male phenotype and genetic quality has been demonstrated in several species (e.g. Houtman 1992; Møller 1994; von Schantz et al. 1999), and in socially monogamous birds, male characteristics may predict both within- and extra-pair mating success (e.g. Kempenaers et al. 1997; Sheldon et al. 1997; Siitari et al. 2002). Extra-pair paternity therefore represents a potentially important means by which female mating preferences can influence variance in male reproductive success, and hence the opportunity for sexual selection (Yezerinac et al. 1995; Sheldon and Ellegren 1999; Byers et al. 2004; Kleven et al. 2006). To understand how female mating decisions influence the evolution of male phenotypic characteristics, it is therefore important to identify the phenotypic cues that females of socially monogamous species use to select extra-pair mates.

As in many passerines, female tree swallows appear to control copulations (e.g. Lifjeld and Robertson 1992); hence, the high rate of extra-pair paternity in this species is likely a reflection of female mating behaviour. Despite numerous investigations, however, the factors influencing female choice of extra-pair mates remain unclear. Results of paired comparisons of extra-pair males and the within-pair males they cuckolded have been inconsistent: some studies have reported no differences (e.g. Dunn et al. 1994), while others have reported that extra-pair males have larger cloacal protuberances (Kempenaers et al. 1999), are in better body condition (Kempenaers et al. 2001) or have longer wings (Stapleton 2005) than within-pair males. These comparisons, however, assume that female tree swallows seek extra-pair copulations with males who are of higher quality relative to their social mates, thereby 'trading up' genetically. In contrast, studies of other passerines have demonstrated that males who sire extra-pair offspring are of consistently higher phenotypic quality than males with no extra-pair fertilization success (unpaired comparisons; e.g. Magrath et al. 2002; Johannessen et al. 2005; Kleven et al. 2005). While this indicates that females seek extra-pair copulations with high-quality males, this comparison does not assume that the probability that a female will seek extra-pair copulations is influenced by the quality of her social mate. It is significant that neither the incidence of extra-pair paternity, nor the proportion of extra-pair offspring in broods of tree swallows are influenced by manipulation of the quality of a female's social mate (Barber et al. 1998). Furthermore, male tree swallows do not reduce their parental investment when the perceived probability of extra-pair paternity in their brood is high (Lifjeld et al. 1993). If the potential cost of extra-pair mating (i.e. loss of paternal care) is low for female tree swallows, this may increase the net benefit of seeking extra-pair copulations, regardless of social mate quality (e.g. different combinations of high-quality genes represented in offspring), thereby maintaining high rates of extra-pair paternity in this species. It is not known, however, if male tree swallows that sire extra-pair offspring are of higher phenotypic quality than males who sire only within-pair offspring in the same breeding population.

In chapter 3, I present the results of a paternity study conducted in 2004 in which I used polymorphic microsatellite loci to assign paternity and, where possible, identify biological fathers of nestlings in 40 broods. I use these data to examine phenotypic correlates of male extra-pair mating success. This allowed me to determine whether females select high-quality males as extra-pair mates, and thus, whether extra-pair mating allows female tree swallows to obtain good genes for their offspring.

1.2.3 Effect of extra-pair paternity on offspring fitness

If mate preferences allow females to obtain good genes for their offspring, mating with preferred males should result in increased offspring fitness. In socially monogamous passerines, genetic benefits of extra-pair mate choice should be evident in comparisons of phenotypic characteristics of within- and extra-pair offspring, since 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). Despite considerable research attention, however, consistent differences between within- and extra-pair offspring of socially monogamous passerines have not been demonstrated (Griffith et al. 2002), and it has therefore been argued that the good genes hypothesis is an inadequate explanation for the existence of multiple mating in these species. 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 within- and extra-pair offspring that are due to paternal genetic contribution may depend on the conditions of the rearing environment. Although context-dependent genetic benefits of mate choice have been demonstrated in a range of taxa (e.g. Jia et al. 2000; Welch 2003), this has only recently been investigated in comparisons of maternal half-siblings in socially monogamous birds (Schmoll et al. 2005). Furthermore, 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; reviewed in Charmantier and Garant 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.

Previous studies examining fitness consequences of extra-pair mating for female tree swallows have not detected phenotypic differences between maternal half-siblings. For example, in a study assessing offspring survival to fledging, Whittingham and Dunn (2001) found no difference in survival between within- and extra-pair young over two breeding seasons. Thus, these authors argued that female tree swallows are not gaining offspring viability benefits through extra-pair fertilizations, at least in terms of viability to fledging. Similarly, Kempenaers et al. (1999) reported no differences in phenotypic quality of maternal half-siblings in this species. Since these studies did not examine the potential influence of environmental conditions on expression of genetic variation within broods, it is not known whether genetic benefits of extra-pair mating in tree swallows are dependent on conditions of the rearing environment. Consequently, in chapter 4, I use paternity data to test the hypothesis that, if female tree swallows seek extra-pair copulations to enhance the genetic quality of offspring, and variation in fitness is conditional, differences in viability of within- and extra-pair young will be dependent on environmental conditions to which nestlings are exposed.

2. Perceived risk of ectoparasitism reduces primary reproductive investment in tree swallows

2.1 Abstract

Female birds may reduce their reproductive investment when paired with a low-quality social mate, or in response to environmental conditions such as infestation of nest-sites by ectoparasites. Nest-dwelling parasite populations increase throughout the breeding season, and can reduce the condition and future survival of both breeding adults and their offspring. Thus, avian hosts should be capable of assessing early cues that predict future ectoparasitism risk, and should either avoid high-risk nest-sites, or facultatively adjust their primary reproductive investment in response to anticipated future costs of parasites. I tested this hypothesis in the tree swallow (*Tachycineta bicolor*), a cavity nesting passerine, by presenting a visual cue of avian fleas on the outer surface of nest boxes. This treatment manipulated perceived ectoparasitism risk without exposing birds to parasites, thereby allowing me to examine facultative responses in the absence of early physiological effects of parasites on female reproductive investment. During one of the study years, birds preferentially occupied control boxes, however, across both years, females nesting in treatment boxes produced significantly smaller clutches, resulting in smaller broods at hatching, relative to those in control boxes. This difference in clutch size could not be explained by differences in phenotypic quality of females or their social mates, indicating that for cavity nesting birds such as tree swallows, the perception of future ectoparasitism risk may be sufficient to induce a facultative reduction in reproductive investment early in the breeding season, before nest-dwelling parasite populations have grown very large.

2.2 Introduction

Nest-dwelling haematophagous ectoparasites have been shown to reduce both reproductive success and condition of breeding birds (Brown et al. 1995, Saino et al. 2002, Nilsson 2003), particularly cavity nesting species (e.g. Oppliger et al. 1994, Fitze et al. 2004). Consequently, many birds exhibit parasite-induced behavioural responses, adjusting timing of breeding (Oppliger et al. 1994), clutch size (Moss and Camin 1970, Heeb et al. 1998), or nestling provisioning rates (Tripet and Richner 1997) in response to infestation of nest-sites by ectoparasites. Many of these responses, however, require reproductive decisions to be made early in the breeding season, before ectoparasite populations have grown very large. Avian hosts are therefore expected to exhibit such responses when, at the onset of breeding, the parasite load of the nest after hatching can be predicted (Johnson and Albrecht 1993, Richner and Heeb 1995). Primary reproductive investment of female birds (e.g. clutch size, egg size) may also be influenced by the quality of their social mate (e.g. Burley 1986; Rintamäki et al. 1998; Gil et al. 2004; Uller et al. 2005). In natural breeding environments, females are faced with variation in both territory and male quality; where territory or nest-site quality has a significant effect on reproductive success, however, choice of a social mate and subsequent reproductive investment by females may be predicted to be more strongly influenced by territory quality than by male quality.

The most reliable predictor of future ectoparasite load is the presence of parasites in the nest at the beginning of the breeding season. Many female birds respond to early parasite exposure by producing smaller clutches (Fitze et al. 2004), or smaller broods at hatching (Oppliger et al. 1994) relative to unexposed females. This reduction in reproductive investment may be a strategic response by females to expected costs of ectoparasitism later in the nesting period, and the associated reduction in perceived reproductive value of offspring produced in infested nest-sites (Johnson and Albrecht 1993). Alternatively, this response may be a direct consequence of early physiological effects of parasites on females, such as reduced body condition or activation of immune defenses (Tschirren et al. 2004). However, previous studies have been unable to distinguish between these mechanisms, since experimental manipulations have typically involved physical exposure of birds to live parasites and the physiological effects thereof. Consequently, the extent to which cavity nesting birds are able to strategically adjust their primary reproductive investment in response to perceived risk of ectoparasitism is not known.

In this study, I investigated whether tree swallows (*Tachycineta bicolor*), an obligate secondary cavity nesting passerine, facultatively reduce their primary reproductive investment in response to a high perceived probability of ectoparasitism by avian fleas (*Ceratophyllus idius*) during the incubation and nestling periods. Fleas are common nest-dwelling parasites of tree swallows (Robertson et al. 1992). Studies of other avian hosts have shown that fleas may affect nestling characteristics (Richner et al. 1993, Nilsson 2003), as well as adult female condition or survival (Brown et al. 1995, Christe et al. 1996, Richner and Tripet 1999), although the effects of fleas on tree swallows remain unclear (Thomas and Shutler 2001, Shutler et al. 2004). I used a visual cue to manipulate perceived ectoparasite risk in nest boxes, without simultaneously exposing females to live parasites; this approach allowed me to examine facultative responses independent from physiological effects of parasites on female reproductive output. Characteristics of resident males were not manipulated; however, I also examined the relative influence of existing variation in male phenotypic quality on female settlement and primary reproductive investment. I discuss my results in the context of host-parasite co-evolution and the adaptive significance of clutch size variation in cavity nesting passerines.

2.3 Methods

2.3.1 Study area and species

I studied two populations of tree swallows located near Prince George (53°N, 122°W), B.C., Canada, during the 2003 and 2004 breeding seasons. Each population was studied during one of the two study years. The study sites are characterized by pasture and wetlands surrounded by second-growth forest of various ages. Birds nest in artificial boxes that are mounted 1.5 m above the ground on fence posts. Nesting material is removed from boxes at the conclusion of each breeding season. Tree swallows have been intensively studied at both sites since 2001. The sites are separated by at least 40 km, and I assume the populations are independent since birds breeding at one site have never been recaptured at the other site in subsequent breeding seasons.

2.3.2 Experimental procedure

As a dispersal mechanism, newly emerged avian fleas (*Ceratophyllus* spp.) aggregate on the outside of nest cavities to seek out hosts (Humphries 1968). This may serve as a visual indicator of both current and future ectoparasite load of potential nest-sites, since it may signal the presence of additional adult fleas in the nest cavity (Harper et al. 1992) as well as developing eggs or larvae. Thus, to manipulate perceived ectoparasite load I mimicked this dispersal behaviour by affixing 20 dead fleas to the outside of experimental nest boxes, beside the entrance hole. This visual display approximated natural flea aggregations previously observed on boxes at both study sites (R. Dawson, pers. comm.). The fleas used in this manipulation had been removed from heat-treated nests collected in the previous breeding season, from the same study areas. The use of dead fleas ensured that experimental boxes exhibited an appropriate visual cue without simultaneously influencing the parasite load, and thus condition, of resident birds. I attached fleas to a 6 x 10 cm piece of white, waterproof paper using spray-on adhesive; this ensured visual detection of fleas by birds inspecting experimental nest boxes. Control boxes had paper coated with adhesive attached in the same position beside the nest box entrance. Papers were affixed to nest boxes at the beginning of the breeding season, prior to male settlement, and left for the duration of the nesting period. Experimental and control conditions were alternately assigned to groups of four nest boxes, distributed throughout each study area. In the 2003 population, this included 24 treatment and 25 control boxes. The 2004 population consisted of 40 treatment and 43 control boxes. Nest boxes were not treated to eliminate living parasites.

I monitored nest boxes throughout the breeding season to determine occupancy, date of clutch initiation, clutch size, brood size at hatching and number of chicks fledged. Once clutches were completed, eggs were individually weighed to the nearest 0.01 g with an electronic scale to determine average and total egg mass. Adult birds were captured in nest boxes using a swing-door trap once hatching was complete. I measured body mass (nearest 0.25 g) with a spring balance. Linear measures of body size, including lengths of the right wing, right ninth primary feather, and tail, were determined to the nearest 0.5 mm using a ruler. To obtain a single measure of body size, I then entered these measurements into a principal component analysis. The first principal component (PC1) explained 68% of the variation in size for females, and 74% for males. All three measurements loaded positively on the PC1, and this component was used in all subsequent analyses of adult body size. Female birds were aged as either second year (SY), or after second year (ASY), based on plumage characteristics (Hussell 1983).

To estimate nestling growth rates, I measured chick mass (nearest 0.125 g using a spring balance) and length of ninth primary (nearest 0.5 mm) every two days, from posthatching day four until day 16. I selected appropriate growth models for body mass and ninth primary using the methods reported by Dawson et al. (2005). I applied the logistic model to describe growth rates of mass, and a linear model for growth of ninth primary. Individual curves were estimated for each nestling within a brood, and the average growth rate constants were used in subsequent comparisons among broods. To determine growth rates of mass, I used all measurements taken from ages four to 16 days. Since ninth primary feathers of most nestlings do not begin to grow until after six days of age, I determined ninth primary growth rates from day eight to day 16. Nestling tree swallows fledge between days 18-22 (Robertson et al. 1992), and can be induced to fledge prematurely if disturbed during this time. Consequently, structural size (length of ninth primary) and body mass at day 16 were used as estimates of fledging size and mass of chicks.

2.3.3 Statistical analysis

If the nest-site treatment increased perceived risk of ectoparasitism, tree swallows may be expected to preferentially settle in control boxes in addition to reducing reproductive investment in treatment boxes. To test this hypothesis, I used a likelihood ratio test to compare proportions of control and experimental boxes that were occupied by breeding birds. To assess primary reproductive investment, I tested the effect of nest-site treatment on clutch size, brood size at hatching, and average egg mass of control and experimental nests using analysis of covariance (ANCOVA). Since female age and clutch initiation date are known to influence clutch size in tree swallows (Robertson et al. 1992), and to account for possible year or site effects between 2003 and 2004, I included in the analysis female age and year (site) as fixed factors, and clutch initiation date as a covariate, in addition to the nest-site treatment.

To examine possible differences in female or male quality that may have accounted for differences in reproductive investment, I used ANCOVA to assess differences in clutch initiation date, body mass and size of resident females, mass and size of their social mates, average nestling growth rates, body mass and size of chicks at fledging, and adult reproductive success (number of chicks fledged). I included nest-site treatment and year (site) as fixed factors in all initial models. In the analyses of female phenotype, I also included female age as a fixed factor, and included initial brood size as a covariate in addition to female age in the analyses of nestling characteristics and number fledged. Effects of factors, covariates and all first-order interactions were initially tested in all ANCOVA models. Variables that did not approach significance ($P > 0.10$) were then removed by a stepwise backward procedure. Adult reproductive success (number of offspring fledged) was only determined for nests fledging at least one young. I also used contingency tables to test whether sub-adult (SY) females were more likely to settle in treatment boxes than older (ASY) females, and whether birds occupying treatment boxes were less likely to fledge at least one young than those nesting in control boxes.

In the 2003 population, three nest-sites (all treatment boxes) were reoccupied by the same female as in a previous breeding season. Since in these cases females may have made investment decisions according to prior knowledge of the nest-site, and less in response to my manipulation during the current breeding season, I conducted all analyses both including and excluding these data. Statistical analyses were performed using SPSS (Norušis 2000), with a significance level of 0.05. Means are presented ± 1 SE.

2.4 Results

In the 2003 population, the visual cue of parasites on the outside of nest boxes reduced the probability of settlement, with eight out of 22 treatment boxes occupied by breeding pairs, compared to 16 out of 24 control boxes ($G = 4.21$, $df = 1$, $P = 0.04$). However, no settlement bias was detected when the three reoccupied boxes were included in the analysis ($G = 2.57$, $df = 1$, $P = 0.11$). No settlement bias was observed in the 2004 population: 21 out of 40 treatment boxes were occupied, compared to 16 of 43 control boxes ($G = 1.97$, $df = 1$, $P = 0.20$).

In all subsequent analyses, results using data from the three reoccupied boxes (see Methods) did not differ from those where these data were excluded; I therefore present results only from those tests where these nests are excluded from the data set. Average egg mass differed between study years ($F_{1,55} = 7.40$, $P < 0.01$), but was not influenced by the nest-site treatment ($F_{1,55} = 1.90$, $P = 0.17$; Table 2.1). However, both the nest-site treatment and clutch initiation date were significant predictors of clutch size (treatment: $F_{1,56} = 4.34$, $P < 0.01$; Table 2.1; initiation date: $F_{1,56} = 12.52$, $P < 0.001$; Fig. 2.1), explaining 40% of the variation in clutch size across both study sites. Thus, although females initiating clutches later in the breeding season laid fewer eggs, birds nesting in treatment boxes produced smaller clutches than those in control boxes across all initiation dates (Fig. 2.1). No additional variation in clutch size was explained by female age or year (site). As with clutch size, brood size at hatching was smaller in treatment boxes (treatment: $F_{1,46} = 6.36$, $P = 0.02$; Table 2.1), as well as in nests that were initiated later in the breeding season (initiation date: $F_{1,46} = 6.67$, $P = 0.01$).

Age distribution of females in treatment and control boxes did not differ (control: 18/31 ASY, treatment: 16/30 ASY; $\chi^2 = 0.14$, $df = 1$, $P = 0.71$). Thus, although older (ASY)

Table 2.1 Comparison of primary reproductive investment of female tree swallows nesting in treatment boxes (displaying fleas), and in control boxes.

	Nest-site manipulation	
Variable	Control	Treatment
Average egg mass (g)		
2003	1.79 ± 0.03 ($n = 13$)	1.79 ± 0.03 ($n = 8$)
2004	1.67 ± 0.03 ($n = 16$)	1.74 ± 0.03 ($n = 21$)
Clutch size**	6.2 ± 0.1 ($n = 32$)	5.7 ± 0.2 ($n = 29$)
Brood size at hatching*	5.7 ± 0.2 ($n = 27$)	5.1 ± 0.2 ($n = 24$)

Note: Means (±1 SE) are presented. * $P < 0.05$, **$P < 0.01$.

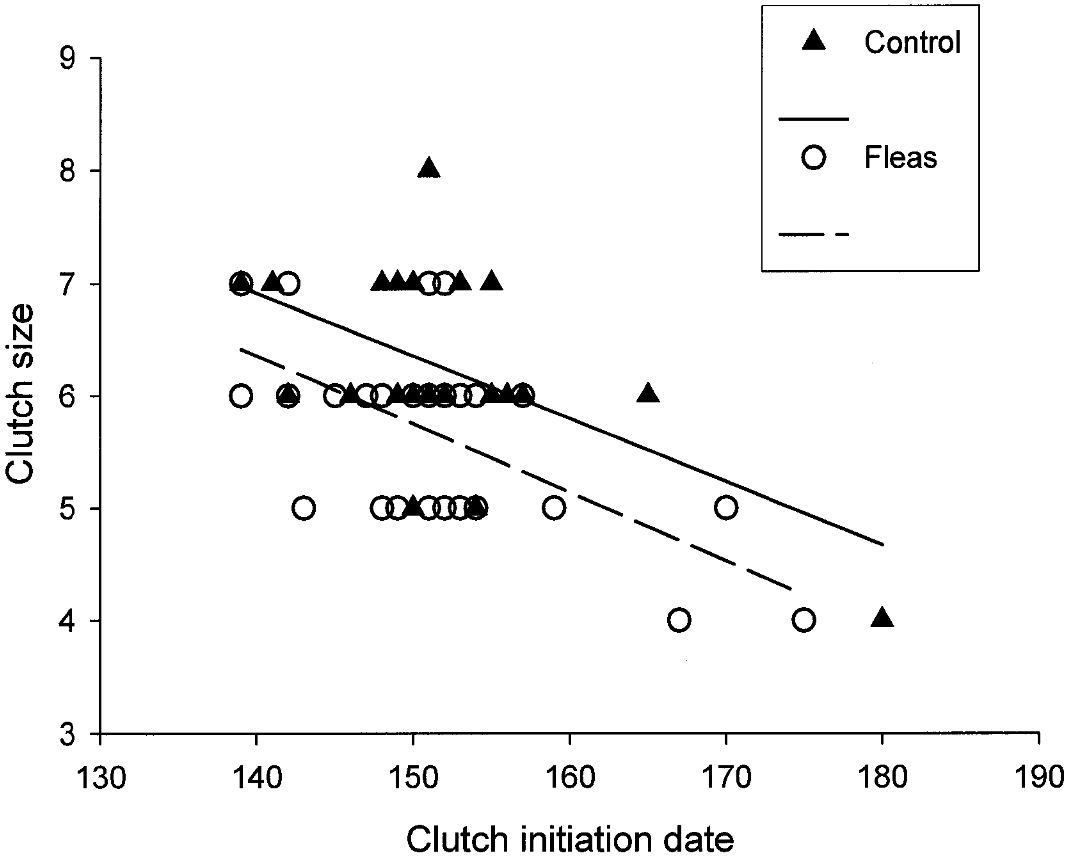

Fig. 2.1 Effect of clutch initiation date (1 = 1 January) and nest-site treatment (visual presentation of fleas on nest boxes) on clutch size of tree swallows. Regression lines describe the decline in clutch size with initiation date for treatment ("fleas") and control nests. Each data point represents one nest. Data from the 2003 and 2004 populations are combined since site (year) was not a significant factor in the ANCOVA model. Some data points have identical values.

females initiated clutches earlier than sub-adult (SY) females ($F_{1,53} = 13.93$, $P < 0.001$), clutch initiation date did not differ between nest-site treatments ($F_{1,53} = 0.43$, $P = 0.51$; Table 2.2). In addition, ASY females were significantly larger than SY females ($F_{1,51} = 11.35$, $P < 0.01$), however there was no difference in size of females occupying treatment and control boxes ($F_{1,51} = 0.16$, $P = 0.69$; Table 2.2). Similarly, female body mass did not differ between nest-site treatment groups ($F_{1,55} = 1.96$, $P = 0.17$; Table 2.2), nor did I detect differences in male phenotype with nest-site treatment (male size: $F_{1,52} = 0.001$, $P = 0.97$; male mass: $F_{1,52} = 1.11$, $P = 0.30$; Table 2.2).

Nestling growth rates did not differ between treatment and control groups (growth of mass: $F_{1,43} = 0.002$, $P = 0.97$; growth of ninth: $F_{1,51} = 0.04$, $P = 0.83$; Table 2.3). Results for length of the ninth primary at 16 days similarly showed no effect of nest-site treatment ($F_{1,43} = 0.01$, $P = 0.92$; Table 2.3). However, length of ninth primaries differed between study years ($F_{1,43} = 52.11$, $P < 0.001$), and nestlings produced by ASY females had significantly longer ninth primaries than those of SY females ($F_{1,43} = 4.84$, $P = 0.03$). Mass of nestlings at 16 days was lower in larger broods ($F_{1,39} = 4.63$, $P = 0.04$), but was not additionally influenced by nest-site treatment ($F_{1,39} = 0.04$, $P = 0.85$; Table 2.3). Among nests that fledged at least one young, the number fledged increased with brood size at hatching ($F_{1,34} = 9.52$, $P < 0.01$) and female age ($F_{1,34} = 4.89$, $P = 0.03$), but did not differ between treatment and control boxes ($F_{1,34} = 1.64$, $P = 0.21$; Table 2.3). There was no difference between treatment and control groups in the probability that nests would fledge at least one young ($\chi^2 = 0.02$, $df = 1$, $P = 0.89$).

Table 2.2 Comparison of clutch initiation date (1 = 1 Jan.) and phenotypic characteristics of adult tree swallows nesting in treatment boxes (displaying fleas), and in control boxes.

	Nest-site manipulation	
Variable	Control	Treatment
Clutch initiation date	152.1 ± 1.3 ($n = 30$)	151.5 ± 1.5 ($n = 29$)
Female mass (g)	19.8 ± 0.3 ($n = 30$)	19.3 ± 0.3 ($n = 27$)
Male mass (g)	19.6 ± 0.2 ($n = 27$)	19.9 ± 0.2 ($n = 27$)
Female PC1 size	0.098 ± 0.2 ($n = 29$)	-0.13 ± 0.2 ($n = 26$)
Male PC1 size	0.060 ± 0.19 ($n = 28$)	0.051 ± 0.16 ($n = 26$)

Note: Means (±1 SE) are presented. Differences between treatments did not approach significance (all *P* values > 0.05; see text for details).

Table 2.3 Comparison of growth and size of tree swallow nestlings, and adult reproductive success in treatment boxes (displaying fleas), and in control boxes.

	Nest-site manipulation	
Variable	Control	Treatment
Mass growth rate	0.58 ± 0.03 ($n = 27$)	0.58 ± 0.02 ($n = 25$)
Ninth primary feather growth rate	4.23 ± 0.09 ($n = 28$)	4.26 ± 0.13 ($n = 25$)
Length of ninth primary at 16 days		
2003 (mm)	36.2 ± 1.9 ($n = 11$)	32.8 ± 4.1 ($n = 4$)
2004 (mm)	46.2 ± 1.0 ($n = 13$)	47.9 ± 1.1 ($n = 20$)
Nestling mass at 16 days (g)	19.7 ± 0.4 ($n = 24$)	20.2 ± 0.4 ($n = 24$)
Number of chicks fledged	4.43 ± 0.23 ($n = 23$)	4.43 ± 0.33 ($n = 21$)

Note: Means (±1 SE) are presented. Differences between treatments did not approach significance (all *P* values > 0.05; see text for details).

2.5 Discussion

Phenotypic characteristics of resident males did not influence primary reproductive investment of their social mates; however, the presence of avian fleas on nest boxes reduced reproductive investment of resident females, with birds in treatment boxes producing smaller clutches than those in control boxes across all clutch initiation dates. Cavity nesting birds have been shown to reduce clutch size when physically exposed to ectoparasites (Moss and Camin 1970, Heeb et al. 1998). In the present study, however, a similar response was observed in birds that had only been exposed to a visual cue indicating a high probability of future ectoparasitism. This supports the hypothesis that the detection of parasites at a nest-site is sufficient to induce cavity nesting birds to facultatively reduce their primary reproductive investment, even in the absence of physical exposure to parasites. Furthermore, these results indicate that investment decisions made by female tree swallows early in the breeding season are more strongly influenced by nest-site quality than by characteristics of their social mate.

The feeding activities of ectoparasites tend to increase resting metabolic rate of nestlings (Nilsson 2003), thereby reducing the size or condition of chicks at fledging (e.g. Richner et al. 1993, Nilsson 2003). Since nestling size commonly predicts survival to the next breeding season (Tinbergen and Boerlijst 1990), chicks produced in nests that have a high probability of current or future ectoparasite infestation would be less likely to survive to breeding age and, thus, would be of lower reproductive value relative to chicks produced in uninfested nests. In addition, nest-dwelling parasites may have direct impacts on female health (Christe et al. 1996) and long-term survival (Brown et al. 1995, Richner and Tripet 1999), and these effects would also reduce the reproductive value of offspring produced in infested nests, since investment in such offspring may reduce future reproductive output of females. Birds occupying infested nest-sites may therefore be predicted to reduce their current reproductive effort in favour of future breeding opportunities. My finding that female tree swallows produce smaller clutches (and therefore smaller broods at hatching) when the probability of future ectoparasitism is high supports this prediction.

By adjusting initial brood size, birds may be capable of indirectly influencing future parasite load of their nests. Larger broods are commonly associated with higher per capita parasite loads (Saino et al. 2002, Shutler et al. 2004, but see Richner et al. 1993). This may be a consequence of reduced cellular immunity of nestlings in large broods (Saino et al. 2002), mediated by nutritional stress and the associated production of glucocorticosteroids (Saino et al. 2003, Franchimont 2004). Growth of parasite populations under these conditions would be enhanced due to the relative inability of nestlings to express anti-parasite immune defenses. In addition, developmental rate of ectoparasites such as fleas is influenced by microclimate conditions in the nest, particularly temperature and humidity (Cotton 1970), and crowding of nestlings in large broods has been suggested to create a microhabitat that may be more favourable for parasite growth and reproduction (Saino et al. 2002). Thus, reduced reproductive investment (i.e. reduced brood size) of cavity nesting birds in response to a high perceived risk of ectoparasitism, as demonstrated in this study, may have the additional consequence of minimizing the potential growth of parasite populations in the nest.

At the beginning of the breeding season, both male and female tree swallows compete aggressively for access to nest-sites (Leffelaar and Robertson 1985, Rendell and Robertson 1989), and this competition may ensure that higher-quality birds are able to occupy the best sites. My nest-site treatment was intended to influence the perception of future parasitism risk, and hence perceived quality of potential nest-sites. It could therefore be argued that higher-quality birds would disproportionately occupy control boxes, and that the observed difference in clutch size in this study was the result of differences in quality of birds nesting in treatment and control boxes. In tree swallows, older females or those in better condition tend to initiate clutches earlier than either second-year females or those in poorer condition (Stutchbury and Robertson 1988, Robertson and Rendell 2001). In this study, neither female age nor clutch initiation date differed between treatment and control nests (Table 2.2). Comparisons of body size and mass of breeding birds similarly showed that quality or condition of birds nesting in treatment boxes did not differ from those in control boxes (Table 2.2). In addition to these comparisons of parental phenotype, analysis of nestling growth rates, as well as size and mass at fledging, indicated no difference in offspring quality (Table 2.3). Finally, despite the initial difference in brood size at hatching, birds nesting in treatment boxes were able to fledge as many young as those in control boxes (Table 2.3). Tree swallows may also adjust clutch size according to phenotypic characteristics other than those I measured, such as individual foraging efficiency (Burness et al. 2001), and it is therefore possible that the observed difference in clutch size in this study reflected phenotypic differences that I was unable to detect. However, any such phenotypic difference underlying variation in clutch size should also be manifested in differential reproductive success; since birds in control boxes did not produce more offspring, nor were their offspring larger or heavier than those of birds occupying treatment boxes, it is unlikely the observed difference in clutch size was due to differences in quality of breeding birds. Moreover, the lack of a treatment effect on number of fledglings produced, despite the initial difference in brood size, suggests that birds in treatment boxes may have been capable of subsequently increasing their investment in nestlings when they did not experience the high flea loads anticipated in treatment nest boxes.

In addition to altering their investment in clutch size, birds in the 2003 population were more likely to settle in control boxes, suggesting they avoided nest-sites with a high perceived risk of ectoparasitism. However, no settlement bias was observed in the 2004 population. Reasons for this discrepancy are not clear, and require additional investigation. Furthermore, control boxes were not consistently occupied earlier than treatment boxes at either site (indicated by similar clutch initiation dates), which suggests other factors may have also influenced nest-site selection. In tree swallows, nest-site choice is strongly limited by intraspecific competition (Leffelaar and Robertson 1985, Rendell and Robertson 1989, Rendell 1993), and in returning adults or recruiting juveniles, may be influenced by previous knowledge of the breeding area (Shutler and Clark 2003). Thus, the nest-site manipulation probably had a minimal influence on settlement patterns at both sites, and appeared to be a more important determinant of primary reproductive investment by birds once they had established a nesting territory.

Whereas many studies have examined the effects of ectoparasites on their avian hosts and the responses of birds to experimental infestation, the mechanisms by which parasites influence host reproductive investment have received less attention. Physical exposure to parasites has direct impacts on breeding birds, and these effects may in turn influence reproductive investment. For example, stimulation of maternal antibody production by parasite exposure enhances the transfer of maternal immunoglobulins to chicks via the egg (Buechler et al. 2002). Strategic adjustments to initial reproductive investment such as reductions in clutch size, however, may be a response to anticipated effects of parasites over the nesting period (Johnson and Albrecht 1993), and may therefore be less dependent on immediate physiological effects of parasite exposure. While continued physical exposure to parasites throughout the nesting period is arguably necessary to influence parental behaviours expressed after clutch formation, such as incubation behaviour and nestling feeding rates, results of this study suggest that in cavity nesting birds, the perception of future ectoparasitism risk may be sufficient to induce a facultative reduction in reproductive investment early in the breeding season, before nest-dwelling parasite populations have grown very large.

3. Male flight feather length predicts extra-pair paternity in an aerial insectivorous passerine

3.1 Abstract

In socially monogamous passerines, extra-pair paternity can increase the variance in male reproductive success, and hence the opportunity for sexual selection. To understand how female mating preferences influence the evolution of male phenotypic characteristics, it is therefore important to identify traits that predict male extra-pair mating success. Tree swallows (*Tachycineta bicolor*) exhibit among the highest rates of extra-pair paternity known to occur in birds, yet it is unclear whether male extra-pair mating success is associated with any morphological traits that may indicate genetic quality. I compared the phenotypic characteristics of male tree swallows who sired extra-pair offspring (EP males) to those who sired only within-pair offspring (WP males) in the same breeding population to test the hypothesis that female tree swallows seek extra-pair copulations to obtain good genes for their offspring. EP males had longer wing and tail feathers, and were more likely to be returning breeders, than WP males. Social mates of EP males also tended to have longer tail feathers and initiate clutches earlier than social mates of WP males, and nestlings in broods of EP males grew their ninth primary feathers faster than nestlings raised in broods of WP males. My results therefore support the hypothesis that female tree swallows engage in extra-pair mating for genetic benefits, and this study is the first to provide a phenotypic basis for female extra-pair mate preference in this species.

3.2 Introduction

Female selection of mates according to phenotypic characteristics that serve as indicators of quality has been well documented (Andersson 1994; reviewed in Jennions and Petrie 2000). Consistent female mate preferences can generate a high variance in male reproductive success, since preferred males mate with more fecund females (Darwin 1871), and in polygynous species, may also attract a greater number of mates (e.g. Gibbs et al. 1990). Among socially monogamous passerines, extra-pair paternity (EPP) represents an additional means by which female mating preferences can influence variance in male reproductive success, and hence the opportunity for sexual selection (Yezerinac et al. 1995; Sheldon and Ellegren 1999; Byers et al. 2004; Kleven et al. 2006) Thus, identification of phenotypic cues that females of socially monogamous species use to select extra-pair mates is important for understanding how female mating decisions influence the evolution of male phenotypic characteristics.

Studies of many socially monogamous birds have identified male characteristics that predict extra-pair mating success, including age (Wagner et al. 1996; Dickinson 2001), song quality (Houtman 1992; Forstmeier et al. 2002), social dominance (Otter et al. 1998), and plumage quality (Saino et al. 1997; Sheldon and Ellegren 1999; Kleven et al. 2006). Males preferred as extra-pair mates may also lose less paternity in their own nests (Webster et al. 1995; Saino et al. 1997), and attract higher-quality social mates (Sheldon and Ellegren 1999; Kleven et al. 2006). Consequently, variance in male reproductive success, and thus, the strength of sexual selection, may be significantly influenced by within-pair as well as extra-pair fertilizations in some species (Webster et al. 1995; Whittingham and Dunn 2005). Other studies, however, have failed to detect phenotypic differences between extra-pair sires and the males they cuckolded (Stutchbury et al. 1997; Webster et al. 2001; Charmantier et al. 2004), and in some cases, extra-pair sires are as likely to lose paternity in their own nests as are other males in the population (e.g. Yezerinac et al. 1995; Kempenaers et al. 1999). Under these conditions, realized within-pair reproductive success of males appears to be unrelated to their phenotypic characteristics, and differential extra-pair fertilization success may be particularly important in determining the strength of sexual selection.

The tree swallow (*Tachycineta bicolor*) is a socially monogamous passerine that exhibits high levels of EPP: up to 90% of broods contain extra-pair offspring, and between 38-69% of nestlings are sired by extra-pair males (Lifjeld et al. 1993; Barber et al. 1996; Whittingham and Dunn 2001). Females do not obtain direct benefits from extra-pair mates; this suggests that EPP provides females with indirect genetic benefits for their offspring. The likelihood of females obtaining extra-pair fertilizations is not influenced by social mate characteristics (Barber et al. 1998), and although there is some evidence that extra-pair males are in better condition or exhibit enhanced sperm production relative to males they cuckolded (Kempenaers et al. 1999, 2001), it is unclear whether male extra-pair mating success is associated with any morphological traits that may indicate genetic quality. EPP is estimated to contribute significantly to the variance in male reproductive success, and hence the opportunity for selection, in tree swallows (Whittingham and Dunn 2005). Understanding the phenotypic basis for extra-pair mate preferences of females is therefore particularly important to determine what traits may be exposed to sexual selection in this species.

In this study, I examined the phenotypic correlates of extra-pair fertilization success in male tree swallows. Most previous studies of EPP in socially monogamous passerines have compared extra-pair males to the resident males they cuckolded, under the assumption that females should select extra-pair mates who are of higher quality relative to their social mate (but see Magrath et al. 2002; Johannessen et al. 2005). That the phenotypic quality of male tree swallows does not appear to influence their within-pair fertilization success, however, may indicate that the benefits of EPP for females are not dependent on phenotypic characteristics of their social mate. In contrast, the high variance in male reproductive success due to EPP in tree swallows suggests that extra-pair fertilization success is not evenly distributed among males in this species, and this would be expected if females are consistent in their preference for high-quality males as extra-pair mates (Webster et al. 1995). I therefore hypothesized that, if female tree swallows select extra-pair mates to obtain good genes for their offspring, and phenotypic characteristics are reliable indicators of genetic quality, males who sire extra-pair offspring (EP males) will be of higher phenotypic quality than resident males who do not sire extra-pair offspring (WP males) in the same breeding population.

3.3 Methods

3.3.1 Study area and field procedures

I studied tree swallows breeding in nest boxes near Prince George BC, Canada (53°N, 123°W) from May to August 2004. The study area consisted of open agricultural fields mixed with patches of coniferous and deciduous forest, and many small wetlands. The site contained 154 nest boxes mounted on fence posts, and placed approximately 25 m apart. Sixty-four boxes were occupied by breeding pairs of tree swallows in 2004.

Beginning in May, I visited nests every one to two days to determine clutch initiation date, clutch size and date of hatch. Adults were captured in nest boxes with a swing-door trap. For each adult, I determined weight using a spring balance (nearest 0.25 g), and measured length of the right wing, ninth primary, and outer rectrix (nearest 0.1 mm with a ruler). These feather length measurements served as indicators of body size, and may also be important measures of quality in adult swallows via their influence on flight performance (e.g. Bowlin and Winkler 2004). Female age was determined as either second year (SY) or after second year (ASY) based on plumage characteristics (Hussell 1983). Unlike females, male tree swallows cannot be aged according to plumage. Instead, males that were captured and banded for the first time as adults were aged as after hatch year (AHY), while recaptured birds were aged according to when they had first been captured as adults (after second year, ASY, after third year, ATY, etc.). At least 90% of breeding males in this population are captured and banded each year, and given the high nest-site fidelity of adult tree swallows (Robertson et al. 1992), this approach allowed me to obtain a reasonable estimate of male age. I banded adults and 14 day old nestlings with standard aluminum leg bands, and collected blood samples (approximately 50 μl) from all birds by puncturing the brachial vein with a 27 gauge needle. Blood samples were stored in 1 ml of Queen's lysis buffer (Seutin et al. 1991) at 4°C prior to DNA extraction (see below).

Parental quality may influence nestling performance, either because one or both parents invest more in provisioning young, or because females allocate more resources to offspring when paired with a high-quality social mate (e.g. Sætre et al. 1995; Gil et al. 1999; Cunningham and Russell 2000). I therefore assessed nestling performance by measuring mass, tarsus and ninth primary feather length of chicks every two days, from ages 4 to 16 days. When nestlings were 12 days old, the T-cell mediated immune response (CMI) was tested by injecting 30 μl 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. I 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 tarsus growth, and a linear model for growth of ninth primary. Model selection procedures followed Dawson et al. (2005). In addition to parental quality, nest-dwelling haematophagous blow fly (*Protocalliphora* spp.) larvae can have significant impacts on nestling performance in a range of bird species, including cavity nesters (e.g. Merino and Potti 1996; O'Brien et al. 2001). To account for variation in nestling characteristics due to intensity of parasitism, nesting material was collected from each box once nestlings had fledged, and was stored in sealed bags at room temperature for 14 days. The nesting material was then sifted to remove all adults and pupae of *Protocalliphora*, and I used the number of pupae per nestling as a relative measure of parasite load in each nest.

To assess parental effort, I measured provisioning rates by placing a digital recorder and small microphone in nest boxes to record begging of chicks. Recordings of at least 1 h (1.31 ± 0.07 h SE, $n = 73$ recordings from 44 nests) were obtained when chicks were 12-14 days old; most nests were recorded on more than one day within this interval to obtain 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 since 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).

3.3.2 Parentage analysis

I isolated genomic DNA by proteinase K-phenol:chloroform extraction, and DNA was subsequently resuspended in TE buffer and stored at -20°C. I used the following three microsatellite primer pairs: 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 µl volumes containing 50-100 ng genomic DNA, 100 µM dNTPs, 2.5-3.0 mM $MgCl_2$, 1x PCR buffer (Invitrogen), 0.2 µM forward (labeled) and reverse primers, and 0.35 units of TAQ DNA polymerase (Invitrogen). PCR reactions were performed using an MJ Research Inc. 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 microsatellite loci used in paternity analysis were highly polymorphic (Table 3.1; details in Appendix I, Figs. A1-A3). The exclusion probability (P_l) for each locus l (with k alleles) was determined with Cervus 2.0 (Marshall et al. 1998), which uses the following formula:

$$P_l = a_1 - 2a_2 + a_3 + 3(a_2a_3 - a_5) - 2(a_2^2 - a_4)$$

where

$$a_n = \sum_{i=1}^{k} p_i^n$$

and p_i is the frequency of allele i, and $a_1 = 1$ (Marshall et al. 1998). The total probability of exclusion (P) across all three loci was then determined by:

$$P = 1 - [(1-P_1)(1-P_2)(1-P_3)]$$

The total exclusion probability for these three loci 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 3.1). Nestlings that shared an allele with their social father at two (37/140; 26%) or three (103/140; 74%) loci were considered within-pair offspring. Of the 37 nestlings mismatching their social father at only one locus, 34 were apparently homozygous at the mismatching locus (HrU6 or HrU10), and only one showed an actual mismatch that was assumed to be due to mutation or a genotyping error (see Fernando et al. 2001). The remaining two were not genotyped at one of the three loci, but matched the social father at the remaining two.

I classified nestlings as extra-pair offspring 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, I 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 extra-pair offspring I identified, 53/76 (70%) mismatched their social father at two loci, and 23/76 (30%) mismatched at all three loci. Males were only assigned extra-pair paternity if their genotype matched the paternally inherited alleles of extra-pair offspring at all three loci. For nestlings that were assigned an extra-pair sire, I calculated the probability that a randomly chosen male would share the same combination of alleles as:

$$\Pi\ (2p_i - p_i^2)$$

Table 3.1 Variability of three microsatellite loci for 111 adult tree swallows.

Locus	Repeat sequence	*n*	Number of alleles	Size range (base pairs)	P_{ei}	h_e	h_o	Null frequency
HrU6	$(AAAG)_{17}(AG)_2(AAAG)_2$	108	55	153-385	0.839	0.919	0.833	0.046
HrU10	$(CTCTT)_{32}$	111	69	209-455	0.956	0.983	0.811	0.094
IBI MP5-29	$(GTTT)_8$	111	6	135-156	0.337	0.612	0.649	0.000

(*n* is the number of unrelated adults typed at each locus, including resident males and females; 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. See text for details of exclusion probability calculation. Repeat sequences are from Primmer et al. 1995, 1996; Crossman 1996.)

where p_i is the frequency of the paternally inherited allele at locus i (Jeffreys et al. 1992). These values ranged from 9.4×10^{-5} to 0.024 (mean ± SD: 0.0033 ± 0.0050, n=32). Details of paternity assignments for each nest are provided in Appendix I, Table A1.

3.3.3 Data analyses

Characteristics of EP and WP males, as well as their social mates, were compared using independent samples *t*-tests. I compared the proportions of EP and WP males paired with ASY females using a likelihood ratio test. Traits such as flight feather length increase with age in adult male tree swallows, particularly between the first year a male is captured as an unbanded resident to his subsequent year as a returning breeder (O'Brien and Dawson, in prep.). Consequently, some phenotypic differences between EP and WP males may reflect differences in age if females select older males as extra-pair mates. Although male tree swallows cannot be aged according to plumage characteristics, high nest-site fidelity of adult tree swallows (Robertson et al. 1992) suggests that unbanded adults are relatively young birds. To test this, I used *t*-tests to compare morphological traits of unbanded (classified as AHY) and recaptured (aged minimally as ASY) males, and I conducted similar comparisons for females known to differ in age based on plumage (SY and ASY). Finally, to assess whether EP males were more likely to be returning breeders than WP males, I used a likelihood ratio test to compare recapture status of males (unbanded or recaptured) in relation to their EPP success (EP or WP).

Analysis of covariance (ANCOVA) was used to assess whether reproductive variables or provisioning rates were predicted by male EPP success (fixed factor: EP or WP). In these analyses, I also included male recapture status and female age (SY or ASY) as fixed factors, as well as ninth primary length of resident birds. In the analyses of clutch size and provisioning rate, clutch initiation date was included as an additional covariate. Factors influencing nestling characteristics (growth rates, CMI response, size and mass at day 16) were also assessed using ANCOVA. Initial models included male EPP success, recapture status and female age as fixed factors, and clutch initiation date, brood size, male and female ninth primary length, and relative *Protocalliphora* abundance as covariates. I initially tested all factors and covariates, as well as all interactions. Terms that did not approach significance ($P > 0.10$) were then removed by backward stepwise elimination until only significant terms remained. Analyses were performed using SPSS (Norušis 2000), and data are presented as mean ± 1 SE unless otherwise indicated. All tests were two-tailed, and results were considered significant at the 0.05 level. Sample sizes are not identical in all analyses due to missing values.

3.4 Results

3.4.1 Patterns of paternity and male reproductive success

Incidence of EPP was similar to other populations of tree swallows. Of the 40 nests included in parentage analysis, 85% (34) contained offspring of mixed paternity. The average proportion of extra-pair offspring in these nests was 0.43 ± 0.04; no nests contained exclusively extra-pair young. In total, 36% (78/218) of all nestlings genotyped were extra-pair offspring, and I identified the biological fathers of 46% (36/78). Of the 34 nests containing offspring of mixed paternity, I identified the biological father of at least one extra-pair offspring in 41% (14) nests. In nine of these nests, all extra-pair young were sired by a single male, while I identified two extra-pair sires in each of four broods, and in one brood I identified three extra-pair sires. I was able to identify the biological father(s) of all nestlings in 32% (11) of the 34 broods with mixed paternity. In total, 17 males were identified as the father of at least one extra-pair offspring, and two of these males each sired extra-pair young in two nests.

Obtaining extra-pair fertilizations did not influence the amount of paternity EP males lost in their own nests (proportion of extra-pair offspring in nests of EP males: 0.41 ± 0.08 *vs.* 0.36 ± 0.05 in nests of WP males; Mann-Whitney *U* test, $U = 107$, $P = 0.61$). Consequently, within-pair reproductive success of EP males (3.1 ± 0.5 young fledged) did not differ from that of WP males (3.5 ± 0.3 young; $t = 0.73$, $df = 29$, $P = 0.47$). Siring extra-pair offspring, however, allowed EP males to improve their total reproductive success (4.8 ± 0.5 young fledged) relative to WP males ($t = 2.24$, $df = 29$, $P = 0.03$).

3.4.2 Extra-pair paternity and characteristics of males and females

EP males had longer flight feathers than WP males (Table 3.2); these differences in feather length reflected male recapture status, since EP males were also more likely to be returning breeders (Table 3.2), and returning breeders had longer flight feathers than unbanded males (Table 3.3). EP males also tended to attract social mates with longer outer rectrices, but were not more likely to be paired with ASY females (Table 3.2). Older females had longer feathers than second year females (Table 3.3), and ASY females initiated egg laying earlier (female age: $F_{1,37} = 4.47$, $P = 0.04$). After accounting for the influence of female age, however, clutch initiation dates for nests of EP males also tended to be earlier (Julian date, where 1 = Jan 1, for EP males: 146.5 ± 0.9; WP males: 148.8 ± 0.8; $F_{1,37} = 3.17$, $P = 0.08$). Neither clutch size nor feeding rates were explained by any of the variables tested (all $P > 0.21$).

Table 3.2 Characteristics of males and their social mates, comparing males that sired extra-pair offspring (EP males) and males that sired only within-pair young (WP males).

Trait	EP males	WP males	Statistic	*P*
Wing length (mm)	121.8 ± 0.7 (17)	120.7 ± 0.5 (23)	$t = 1.25$	0.22
Ninth primary length (mm)	95.6 ± 0.6 (17)	93.9 ± 0.6 (23)	$t = 2.06$	0.046
Outer rectrix length (mm)	58.1 ± 0.6 (17)	56.5 ± 0.3 (23)	$t = 2.49$	0.02
Body mass (g)	19.57 ± 0.22 (17)	19.59 ± 0.22 (23)	$t = -0.04$	0.97
% returning breeders[a]	59 (17)	17 (23)	$G = 7.51$	<0.01
Social mates[b]				
Wing length (mm)	115.6 ± 0.5 (17)	115.9 ± 0.5 (23)	$t = -0.42$	0.68
Ninth primary length (mm)	90.2 ± 0.7 (17)	89.4 ± 0.5 (23)	$t = 1.02$	0.32
Outer rectrix length (mm)	56.2 ± 0.5 (17)	55.1 ± 0.3 (23)	$t = 2.02$	0.05
Body mass (g)	20.17 ± 0.21 (16)	19.80 ± 0.23 (23)	$t = 1.13$	0.26
% ASY[c]	76 (17)	65 (23)	$G = 0.60$	0.44

Data are mean ± SE (sample size).

[a] Returning breeder = Banded male that was captured as a resident in a previous breeding season.

[b] Females that were paired with EP and WP males.

[c] ASY = After second year, based on plumage color (Hussell 1983).

Table 3.3 Comparison of unbanded males and returning breeders[a], as well as second year (SY) and after second year (ASY) females[b].

Trait	Unbanded males	Banded males	*t*	*P*
Wing length (mm)	120.7 ± 0.5 (26)	122.1 ± 0.8 (14)	1.63	0.11
Ninth primary length (mm)	93.8 ± 0.5 (26)	96.1 ± 0.7 (14)	2.63	0.01
Outer rectrix length (mm)	56.4 ± 0.4 (26)	58.6 ± 0.4 (14)	3.61	0.001
Body mass (g)	19.48 ± 0.20 (26)	19.77 ± 0.26 (14)	0.87	0.39
	SY females	ASY females		
Wing length (mm)	114.2 ± 0.5 (14)	116.5 ± 0.4 (34)	3.38	0.001
Ninth primary length (mm)	87.3 ± 0.7 (14)	90.6 ± 0.4 (34)	4.48	<0.001
Outer rectrix length (mm)	54.4 ± 0.5 (15)	56.1 ± 0.3 (34)	3.33	0.002
Body mass (g)	19.67 ± 0.28 (15)	19.90 ± 0.19 (33)	0.70	0.49

Data are mean ± SE (sample size).

[a] Returning breeder = Banded male that was captured as a resident in a previous breeding season (aged as minimally ASY).

[b] Female age based on plumage color (Hussell 1983).

3.4.3 Nestling performance

Nestlings grew their ninth primary feathers faster when exposed to fewer *Protocalliphora* larvae ($F_{1,32} = 10.88$, $P = 0.002$; Fig. 3.1), but across all levels of parasite infestation, ninth primary growth rates were higher for nestlings raised in broods of EP sires ($F_{1,32} = 5.54$, $P = 0.025$; Fig. 3.1). Characteristics of the social father did not influence nestling growth of mass or tarsus (all $P > 0.12$), however, offspring of females with longer ninth primary feathers grew their tarsi faster ($F_{1,40} = 9.64$, $P = 0.003$). Mass of nestlings at day 16 was not influenced by any variables tested (all $P > 0.20$), whereas size of nestlings at day 16, as well as CMI response, was influenced only by *Protocalliphora* abundance: chicks exposed to fewer parasites had longer ninth primary feathers ($F_{1,26} = 7.44$, $P = 0.01$), marginally longer tarsi ($F_{1,26} = 3.70$, $P = 0.07$), and exhibited a greater CMI response ($F_{1,34} = 6.72$, $P = 0.01$).

3.5 Discussion

According to the 'good genes' hypothesis, females can assess the genetic quality of males based on phenotypic cues, and should seek extra-pair copulations with males of high genetic quality (Andersson 1994). Tree swallows exhibit among the highest rates of EPP identified in socially monogamous birds, yet it is unclear what, if any, phenotypic traits females use to assess potential extra-pair mates. Previous studies of this species have identified few consistent differences in paired comparisons of extra-pair sires and the males they cuckolded (Dunn et al. 1994; Kempenaers et al. 1999; Kempenaers et al. 2001), indicating that female tree swallows do not necessarily 'trade up' by selecting extra-pair mates who are of higher phenotypic quality relative to their social mate. Furthermore, neither

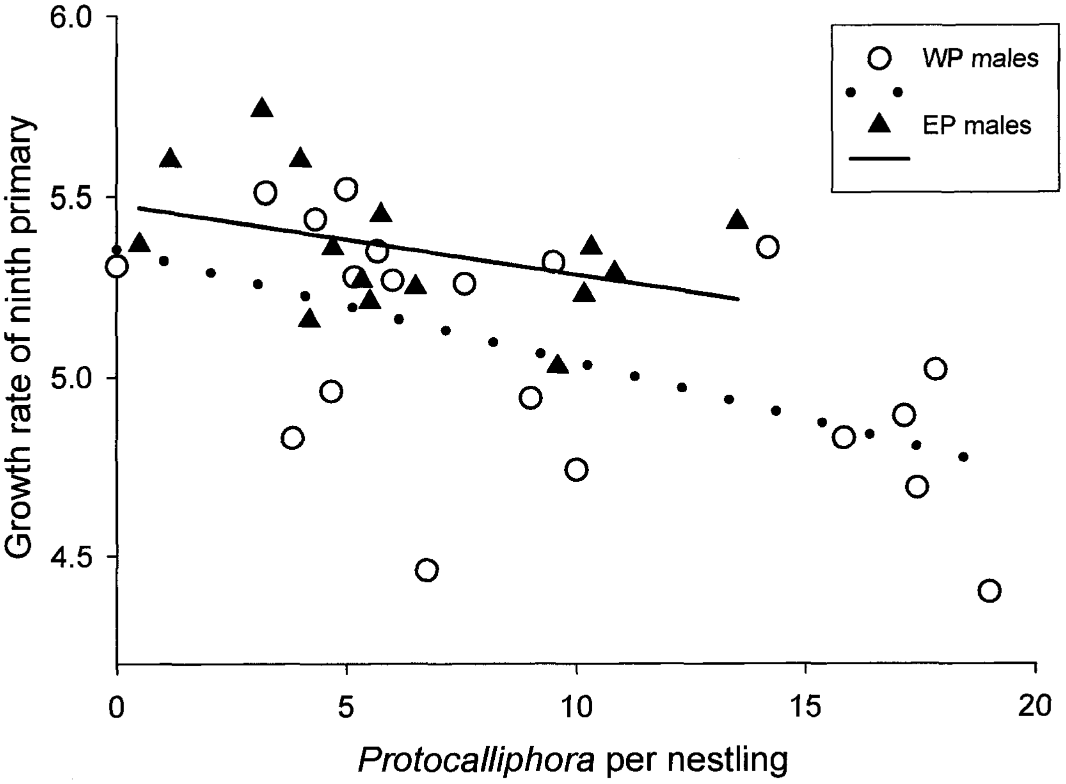

Fig. 3.1 The influence of *Protocalliphora* abundance on growth rates of ninth primary feathers for nestlings in broods of males who sired extra-pair offspring (EP males) and males who sired only within-pair offspring (WP males) in the same breeding population.

the presence nor the proportion of extra-pair offspring in a brood is related to the phenotypic characteristics of the social father (Barber et al. 1998), suggesting that the benefits of EPP for female tree swallows are not necessarily dependent on social mate quality.

Results of this study indicate that female tree swallows select extra-pair mates of high absolute phenotypic quality, since male tree swallows that sired extra-pair offspring had longer flight feathers compared to males with no extra-pair success. A longitudinal analysis of this population has shown that flight feather length increases with male age (O'Brien and Dawson, in prep.), and in the present study, EP males were more likely to be returning breeders (Table 3.2). Female preferences for long-lived males as extra-pair mates have been demonstrated in other bird species (e.g. Weatherhead and Boag 1995; Wagner et al. 1996; Dickinson 2001; Kleven et al. 2005); to the extent that flight feather length signals age of male tree swallows, selection of extra-pair mates with longer flight feathers would similarly allow females to select genes associated with longevity. Alternatively, longer feathers may improve flight performance and foraging ability in aerial insectivores such as tree swallows (Bowlin and Winkler 2004). If the ability to allocate resources to feather growth is heritable, this trait may also have direct fitness consequences independent of age. Results of this study therefore support the hypothesis that extra-pair mating in tree swallows allows females to obtain good genes for their offspring, and provide a potential phenotypic basis for female extra-pair mate preference that may account for the high estimated variance in male reproductive success due to EPP in this species.

Theory suggests that preferred males should acquire high-quality social mates (Darwin 1871) in addition to obtaining extra-pair fertilizations. High-quality female birds initiate breeding earlier (Price et al. 1988), and female tree swallows that breed early in the season produce larger clutches (Robertson et al. 1992), exhibit superior foraging efficiency

(Bowlin and Winkler 2004) and stronger immune responses (Ardia 2005) compared to late-breeding birds. Flight feather length of females may reflect maternal foraging ability or experience (Bowlin and Winkler 2004), as well as age (Table 3.3, this study), and may therefore be an important measure of quality. In this study, EP males were mated to females who tended to breed earlier and had marginally longer outer tail feathers relative to social mates of WP males. This may be either due to congruence in arrival time of EP males and their social mates, or a direct consequence of female social mate preference; however, these differences further suggest that EP males are of higher quality than WP males and, hence, that female tree swallows select extra-pair mates for genetic benefits.

Nestlings raised in broods of EP males grew their ninth primary feathers faster (Fig. 3.1), despite the finding that provisioning rates did not differ between nests of EP and WP males. There are several possible explanations for this. First, it is possible that parental provisioning effort in nests of EP males was greater, but this was not reflected in feeding rates (e.g. food quality or load size; see Sætre et al. 1995; but see McCarty 2002). Furthermore, in many bird species, females paired with high-quality males increase their reproductive investment (Burley 1986) by increasing egg size (Uller et al. 2005), clutch size (Rintamäki et al. 1998), yolk androgens (Gil et al. 2004), or parental effort (Limbourg et al. 2004). In this study, if EP males were of higher quality, differential allocation by their social mates, whether by feeding behavior or clutch investment, may have resulted in enhanced nestling performance. Finally, the ability to preferentially allocate resources to growth of flight feathers may be heritable; in broods of EP males, both within- and extra-pair offspring may therefore exhibit relatively rapid feather growth, since both may benefit from inheriting high-quality paternal genes. Further investigation is needed to distinguish among these explanations for the observed differences in feather growth rates of nestlings in this study.

Regardless of the mechanism, however, these results provide additional evidence for the hypothesis that EP males are of higher quality than males that do not sire extra-pair young in the same breeding population and, thus, that female tree swallows engage in extra-pair mating to obtain good genes for their offspring.

The good genes hypothesis of mate choice predicts that females mated to high-quality males should be less likely to seek extra-pair copulations than females paired with low-quality males (Jennions and Petrie 2000). In tree swallows, however, no characteristics of males have been identified that predict either the probability of losing paternity, or the proportion of extra-pair young, in their own nests (Barber et al. 1998; Kempenaers et al. 2001). This suggests that the probability that a female will seek extra-pair copulations is not dependent on the characteristics of her social mate. Recent evidence indicates that the genetic benefits of extra-pair mate choice may depend on the environmental conditions to which nestlings are exposed (Schmoll et al. 2005; see also Chapter 4). If these conditions are unpredictable early in the breeding season, females paired with high-quality males may not be assured of obtaining fitness benefits by investing only in within-pair mating, and seeking extra-pair copulations with other high-quality males may represent a conservative genetic 'bet-hedging' strategy (Yasui 1998). Under these conditions, males that sire extra-pair offspring should be of consistently high quality, but individual extra-pair and within-pair mating success of males will not necessarily be correlated. Results of this study demonstrate that, in tree swallows, EP males indeed appear to be of higher phenotypic quality, as they possess longer flight feathers, and are more likely to be returning breeders (and may therefore be older), than WP males. Furthermore, EP males did not lose less paternity in their own nests, indicating that female tree swallows may use extra-pair mating as a genetic bet-hedging strategy, irrespective of the quality of their social mate. This latter observation also indicates that male phenotypic traits associated with extra-pair mating success (e.g. flight feather length) do not covary with functional fertility (Sheldon 1994), and consequently reflect female mating preferences based on genetic quality rather than fertility assurance.

Taken together, results of this investigation indicate that male tree swallows who obtain extra-pair fertilizations in my study population appear to be of high quality, which supports the hypothesis that female swallows seek extra-pair copulations to obtain good genes for their offspring. Moreover, since EP males did not lose more paternity in their own nests than males who sired only within-pair offspring, their total reproductive success (number of fledglings produced, including extra-pair offspring) was greater than that of WP males. The opportunity for sexual selection via extra-pair fertilizations in this species may therefore be considerable. As shown in this study, differences in male quality may not necessarily be reflected in differential within-pair mating success, and studies that fail to demonstrate such effects, particularly for species in which incidence of extra-pair paternity is high, should also consider relative extra-pair mating success of males in relation to phenotypic quality (e.g. Johnsen et al. 2001). Finally, there is increasing evidence that phenotypic quality of males improves with age (e.g. Garamszegi et al. 2005) or that particular traits are correlated with male longevity (e.g. Forstmeier et al. 2006). My results suggest that female tree swallows may select older males as extra-pair mates; more longitudinal studies are needed, however, to determine the extent to which flight feather length in tree swallows varies independent of age, and thus, whether flight feather length has the potential to be a sexually selected trait via EPP in this species.

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

4.1 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. I tested the hypothesis that genetic benefits of extra-pair mating are environment-dependent by comparing phenotypic characteristics of WPO and EPO in the tree swallow (*Tachycineta bicolor*) in relation to experimentally modified nest temperature and natural abundance of haematophagous parasites (*Protocalliphora* spp.). EPO grew their ninth primary feathers faster than WPO, and tended to grow mass faster, under all conditions. EPO also tended to have longer ninth primary feathers than WPO at fledging in all rearing environments, but they consistently exceeded their maternal half-siblings in this trait under favourable conditions, when parasite abundance was low and they were positioned early in the hatching sequence. 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.

4.2 Introduction

Extra-pair paternity is common in socially monogamous passerines (Petrie and Kempenaers 1998), and since 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 better condition (Sheldon et al. 1997) or exhibit enhanced immune responses (Johnsen 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, since 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 contribution may depend on the conditions of the rearing environment. Context-dependence of the 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 my knowledge, however, only one study has considered context-dependence in comparisons of WPO and EPO of socially monogamous birds: in coal tits (*Parus ater*), EPO that hatch from nests initiated late in the breeding season, when food is less abundant, have higher recruitment rates, while WPO hatching from early nests, when food stress is lower, are more likely to recruit (Schmoll et al. 2005). These results suggest that genetic benefits of extra-pair mating are evident under high stress conditions; however, it is unclear whether context-dependent genetic benefits of mate choice should be consistently evident in low or high stress environments (e.g. Welch 2003; reviewed in Charmantier and Garant 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.

I compared phenotypic characteristics of maternal half-siblings in nests of tree swallows (*Tachycineta bicolor*) exposed to varying environmental conditions. 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. 2005). 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. 2005). In addition, nest-dwelling 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). I therefore tested the effects of experimentally manipulated nest temperature, as well as the naturally occurring abundance of *Protocalliphora* spp., on relative performance of WPO and EPO. If female tree swallows engage in extra-pair mating to obtain genetic benefits for their offspring, and these benefits are context-dependent, I 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.

4.3 Methods

4.3.1 Study area and study species

I studied tree swallows breeding in nest boxes near Prince George BC, Canada (53°N, 123°W) from May to August 2004. The study area consisted of open agricultural fields mixed with patches of coniferous and deciduous forest, and many small wetlands. The site contained 154 nest boxes mounted on fence posts, and placed approximately 25 m apart. Sixty-four boxes were occupied by breeding pairs of tree swallows in 2004.

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).

4.3.2 Field and experimental methods

Beginning in May, nests were visited every one to two days to determine clutch initiation date, clutch size and date of hatch. Adults were captured in nest boxes with a swing-door trap. I banded adults and 14 day old nestlings with standard aluminum 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. (2005), I experimentally increased temperature of treatment nests by 5-10°C by installing small heating pads in nests when young were between 4 and 16 days of age. These pads, which were changed every two days, produced heat when the iron powder they contained was exposed to air and oxidized. Control nests contained pads in which the iron powder had been fully oxidized, so heat was not produced. Treatment and control nests corresponded to 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. The nesting material was then sifted to remove all adults and pupae of *Protocalliphora*. Since the abundance of *Protocalliphora* increased with brood size (see Results), I 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. I 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 (1.31 ± 0.07 h SE, $n = 73$ recordings from 44 nests) were obtained when chicks were 12-14 days old; most nests were recorded on more than one day within this interval to obtain 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 since 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).

I measured nestling mass, tarsus and ninth primary feather length every two days, from ages 4 to 16 days. When chicks were 12 days old, the T-cell mediated immune response (CMI) was tested by injecting 30 μl 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. I 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 tarsus growth, and a linear model for growth of ninth primary. Model selection procedures followed Dawson et al. (2005).

4.3.3 Parentage analysis

Blood samples were stored in 1 ml of Queen's lysis buffer (Seutin et al. 1991) at 4°C. I isolated genomic DNA by proteinase K-phenol:chloroform extraction, and DNA was subsequently resuspended in TE buffer and stored at -20°C. I 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 procedures and reaction conditions were as described in Chapter 3 (section 3.3.2). PCR products were analysed using a Beckman-Coulter CEQ 8000 automated sequencer.

Two of the three loci were highly polymorphic (Table 3.1), and the exclusion probability for all three loci (Jamieson 1994) was 0.995 with one parent known. Paternity assignment procedures are described in detail in Chapter 3 (3.3.2). Briefly, I 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 at two loci, however, I 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 that I 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 (37/140; 26%) or three (103/140; 74%) loci were considered WPO.

4.3.4 Statistical analysis

I used paired *t*-tests to compare mean values of phenotypic traits for EPO and WPO in each nest. This allowed me 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, I used analysis of covariance (ANCOVA) to test for effects of the heating treatment as a fixed factor, and *Protocalliphora* abundance (number of pupae/nestling), 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 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, I therefore included difference in mass at day four (average mass of EPO-average mass of WPO) as an additional covariate representing relative hatch order. For each phenotypic trait, I 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, while values < 0 indicate the opposite trend. Initial models tested all main effects and interactions. Terms that did not approach significance ($P > 0.10$) were then removed by backward stepwise elimination until only significant terms remained, or a significant interaction was detected. I used SPSS (Norušis 2000) software for all analyses. Means are presented ± 1 standard error (SE) and results were considered significant at the 0.05 level.

4.4 Results

Incidence of extra-pair paternity was similar to other populations of tree swallows. Of the 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, 78/218 (36%) of all offspring genotyped were EPO. Fledging success across all nests 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 (65/78, 83% EPO *vs.* 114/141, 81% WPO; $G = 0.77$, $df = 1$, $P = 0.38$), nor were they more likely to hatch first (EPO heavier at day 4: 17 nests *vs.* WPO heavier: 14 nests; $G = 0.29$, $df = 1$, $P = 0.59$). Extra-pair young grew their ninth primary feathers faster, and tended to exhibit faster growth rates of mass, than their within-pair half-siblings (Table 4.1). EPO also tended to have longer ninth primary feathers at fledging, but did not consistently differ from WPO in any other phenotypic measures (Table 4.1).

Nests with larger broods contained more *Protocalliphora* pupae ($r = 0.34$, $P = 0.03$, $n = 43$), 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 $P > 0.10$). In the analysis of difference in

Table 4.1 Results of paired *t*-tests comparing phenotypic traits of extra-pair and within-pair offspring of tree swallows. Positive values indicate that extra-pair offspring were larger or heavier than within-pair offspring in the same nest.

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

length of ninth primary at day 16, however, I detected a significant interaction between relative hatch order of maternal half-siblings and level of parasite infestation ($F_{1,23} = 12.91$, $P = 0.002$). When I 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 at fledging, with EPO having longer primaries relative to WPO under low to moderate levels of infestation ($F_{1,13} = 7.68$, $P = 0.02$; Fig. 4.1). 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 ($F_{1,13} = 2.76$, $P = 0.12$). In nests where WPO hatched earlier, however, neither *Protocalliphora* abundance ($F_{1,8} = 0.54$, $P = 0.48$; Fig. 4.1) nor degree of hatching asynchrony ($F_{1,8} = 2.00$, $P = 0.20$) affected relative chick performance. None of the variables I measured influenced differences in mass or tarsus of EPO and WPO at day 16, or relative CMI response (all $P > 0.14$). Difference in tarsus growth rate was related only to hatch order: nestlings positioned early in the hatching sequence 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 ($r = 0.35$, $P = 0.03$, $n = 39$), however, provisioning rate was not a significant predictor of relative nestling performance in any of the above analyses (all $P > 0.28$).

4.5 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. My results support this

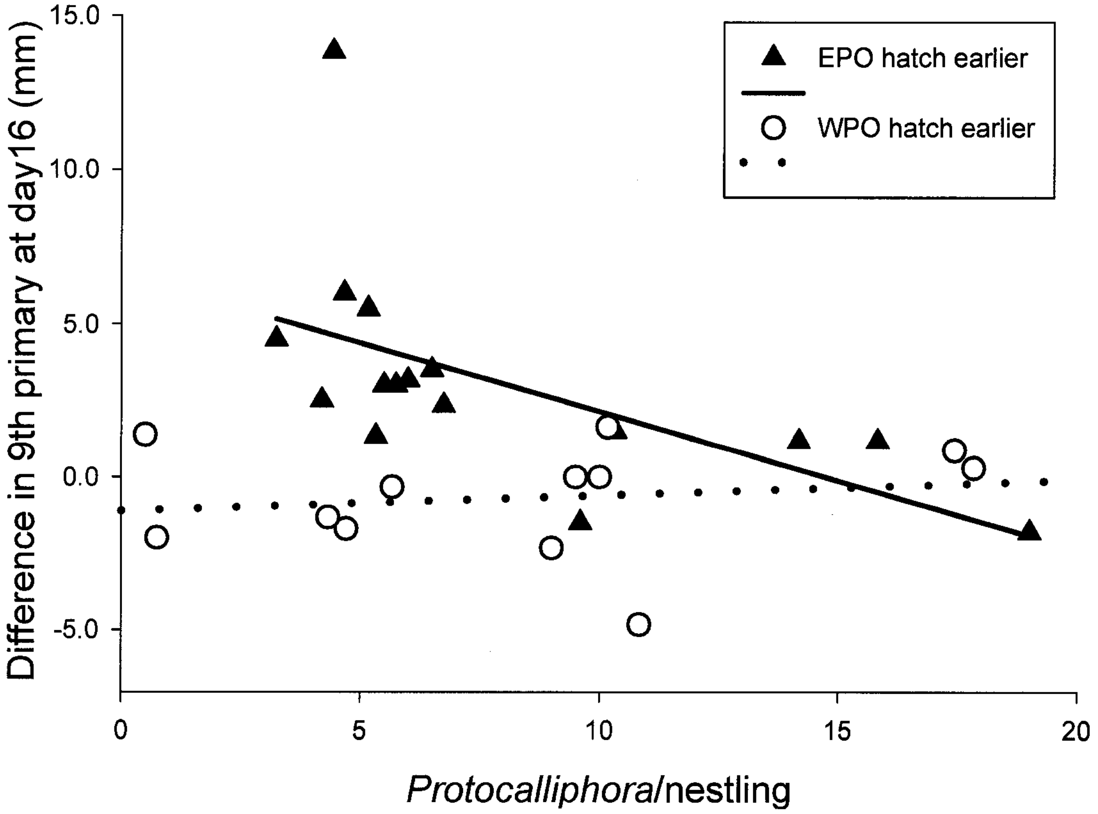

Fig. 4.1 Difference in length of ninth primary at day 16 between extra-pair offspring (EPO) and within-pair offspring (WPO) of tree swallows in relation to parasite (*Protocalliphora* spp.) abundance and position of nestlings in the hatching sequence. Differences were calculated as mean value for EPO – 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 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.

hypothesis, since 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, while WPO that hatched early did not show a similar advantage under low parasite loads (Fig. 4.1). 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 tend to attract social mates of higher quality and are more likely to sire extra-pair young (Chapter 3). 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 (Hoffmann and Merilä 1999). Studies of heritability (Qvarnström 1999) and mate choice (Welch 2003) have supported this prediction, and the results of this 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 (Hoffmann 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 conditions, 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, whereas phenotypic differences between EPO and WPO were evident when parasite abundance was low. This suggests that stresses due to environmental factors such as feeding conditions or nest temperature 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 halfsibs (Table 4.1), and under favourable conditions, had considerably longer ninth primaries at fledging (Fig. 4.1). This suggests that EPO had a greater tendency to preferentially allocate resources to wing growth than WPO in my 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). I am 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). Since 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 maternal 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. 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.

5. General Discussion

Female mate preference has long been recognized as an important factor influencing the evolution of male phenotypic characteristics (Darwin 1871; Andersson 1994). This has generated considerable research interest in identifying traits that predict male reproductive success, and which may therefore be subject to sexual selection (reviewed in Jennions and Petrie 1997). The discovery of high rates of extra-pair paternity in broods of socially monogamous birds (reviewed in Griffith et al. 2002) reveals that variance in male reproductive success, and consequently the strength of sexual selection, may be influenced by a combination of within- and extra-pair paternity (Møller 1992; Byers et al. 2004; Whittingham and Dunn 2005), depending in part on the extent to which male phenotypic quality influences within- and extra-pair mating success. The objective of this study was to examine factors influencing female selection of social and extra-pair mates in the tree swallow, a socially monogamous passerine that exhibits high rates of extra-pair paternity. I also assessed the potential fitness benefits of extra-pair mating for females by determining the influence of paternal genetic contribution on relative performance of maternal half-siblings prior to fledging.

Reproductive success of secondary cavity nesting birds may be limited by availability and quality of nest-sites (Holroyd 1975; Rendell and Robertson 1989; Li and Martin 1991). Male quality generally covaries with the quality of resources (e.g. territory or nest-site) they defend (Searcy 1982), and it is therefore difficult to distinguish between the importance of male and nest-site quality for female selection of social mates. Studies that attempted to dissociate male and nest-site quality in pied flycatchers (*Ficedula hypoleuca*) have demonstrated that female settlement decisions are more strongly influenced by quality of nest-sites than resident males (Alatalo et al. 1986; Slagsvold 1986). In Chapter 2, I showed that nest-site characteristics may similarly influence female settlement decisions in tree swallows, although this tendency was not evident in both study populations. I am unable to provide an explanation for this difference, but recommend that future investigations assess variation in nest-site selectivity of females in relation to cavity availability among populations of tree swallows. Furthermore, since male quality was not manipulated in this study, and due to the difficulty of capturing male tree swallows early in the breeding season, it is not known whether males who successfully attract social mates are of higher quality than males who remain unpaired. Despite the logistic constraints, this question merits further investigation.

Female birds may adjust clutch size according to their provisioning ability or body condition (e.g. Slagsvold and Lifjeld 1988, 1990; Bowlin and Winkler 2004), the quality of their social mate (Rintamäki et al. 1998), or environmental factors such as the presence of nest-dwelling ectoparasites (Moss and Camin 1970; Heeb et al. 1998). In this study, the manipulation of perceived risk of ectoparasitism was associated with a reduction in clutch size of tree swallows in both study populations (Chapter 2). This effect could not be accounted for by phenotypic quality of females or their social mates, indicating that nest-site characteristics, notably the presence of ectoparasites, may have a particularly important influence on primary reproductive investment decisions in secondary cavity nesting birds such as tree swallows. These results, combined with the inconsistent settlement effect of the nest-site manipulation, highlight the possibility that obligate secondary cavity nesters, due to intense competition for nest-sites or mates, may be constrained from rejecting sites of potentially low-quality (see also Nilsson 1984; Dale et al. 1992; Dale and Slagsvold 1996). Adjustment of primary reproductive investment after settlement may instead allow females to secure a breeding opportunity, while still responding to the perceived reproductive value of offspring produced in the current breeding attempt.

In the majority of socially monogamous species, extra-pair mating is not associated with any direct benefits for females (e.g. parental care by extra-pair males; Li and Brown 2002), and selection of extra-pair mates is therefore expected to provide females with indirect genetic benefits (Neff and Pitcher 2005). A number of studies have identified male characteristics that predict extra-pair mating success (e.g. song or plumage quality; Forstmeier et al. 2002; Kleven et al. 2006) and offspring quality (Møller 1994; Hasselquist et al. 1996), suggesting that female birds seek extra-pair copulations to obtain good genes for their offspring. Male tree swallows who sired extra-pair offspring had longer flight feathers, and were more likely to be returning breeders, compared to resident males who did not sire extra-pair offspring in my study population (Chapter 3). These results could be interpreted as reflecting post-copulatory mechanisms such as differential fertilization success among males, if fertilization success is correlated with phenotypic quality (see Sheldon 1994); however, one would then also expect EP males to lose less paternity in their own nests, which was not the case. Consequently, this study provides evidence that female tree swallows select high-quality, and possibly older, males as extra-pair mates, which is consistent with the hypothesis that extra-pair fertilizations in this species allow females to obtain good genes for their offspring.

There is strong evidence that flight feather length increases with age in both males (O'Brien and Dawson, in prep.) and females (Table 3.3) in my study population. Longitudinal studies are needed to determine whether flight feather length of males has the potential to be a sexually selected character in tree swallows, since this will depend on the extent to which feather length varies independent of age. Furthermore, it will be important to assess the display function of flight feather length in this species: flight performance of females is associated with wing loading (Bowlin and Winkler 2004), which is partly a function of the length of primary feathers; since this is likely the case for males as well, my finding that extra-pair males had longer flight feathers (Chapter 3) may indicate that female tree swallows assess potential extra-pair mates according to the quality of their aerial displays.

Previous studies of tree swallows have not detected consistent phenotypic differences between extra-pair males and the resident males they cuckolded (e.g. Lifjeld et al. 1993; Kempenaers et al. 2001), suggesting that females do not necessarily compare the quality of their social mate to that of potential extra-pair mates. Consequently, the phenotypic quality of a male does not predict his within-pair reproductive success: females paired with high-quality males appear to be as likely to seek extra-pair copulations as females paired with lower-quality males (Chapter 3; see also Barber et al. 1998). This is not necessarily incompatible with the good genes hypothesis, however; it is known that phenotypic expression of heritable variation is influenced by environmental conditions (e.g. Merilä 1997), and my results suggest that genetic benefits of extra-pair mate choice in tree swallows may similarly be dependent on the environmental conditions to which nestlings are exposed (Chapter 4). Extra-pair mating in this species may therefore function as a form of genetic ‘bet-hedging’ in response to genotype-environment interactions in an unpredictable environment, regardless of social mate characteristics. It is intriguing that extra-pair offspring had longer ninth primaries at fledging only when the prevalence of haematophagous parasites (*Protocalliphora* spp.) was low (Chapter 4, Fig. 4.1). Although parasite loads were not manipulated, this suggests that parasites may be an important environmental stressor limiting the expression of genetic benefits of mate choice in my study population of tree swallows.

Additional studies are necessary to examine this hypothesis in more detail, and to determine whether similar effects are evident in other populations, as well as other bird species. It is apparent from my results, however, that genetic benefits of mate choice, and the evolution of female mate preferences, may be influenced by more complex mechanisms than many previous investigations have assumed.

Considerable research attention has been directed toward understanding the factors influencing mate choice by female tree swallows, in part due to the high rates of extra-pair paternity that have been identified in this species. Interestingly, all attempts to identify extra-pair males, including the present study, have been unable to identify a large proportion of extra-pair sires. Consequently, several persistent questions remain unanswered for this species, including: who are the remaining extra-pair sires, and where do they obtain extra-pair copulations? There is evidence that some males may exhibit a "floater" strategy, by which they forego nesting and invest exclusively in extra-pair copulations (Kempenaers et al. 2001). Results of this study show that older males are more successful at obtaining extra-pair fertilizations; this may suggest that floating represents an age-dependent reproductive strategy in male tree swallows, with males being more likely to adopt this strategy as they age. Tests of this hypothesis will require detailed longitudinal studies that follow individual males over successive breeding seasons. It may also be important to recognize that, if female tree swallows follow a general strategy of selecting older males in the breeding population as extra-pair mates (Chapter 3), detection of phenotypic differences via paired comparisons of extra-pair males and the resident males they cuckold may be dependent on the age structure of the population. For example, if resident males are on average older in a given year, there may be no detectable phenotypic differences in paired comparisons with extra-pair sires, whereas in years where more young males recruit as residents, extra-pair sires may, on average, be phenotypically distinguishable from cuckolded residents. Discrepancies in results of such comparisons among years, or among breeding populations of tree swallows, may therefore be attributed to differences in population age structure, although this has not previously been considered.

My results indicate that EP males have longer flight feathers (primaries and outer rectrices) relative to males that do not sire EPO. Since these traits are associated with male age in my study population (Chapter 3), and were not manipulated in my study, I am unable to determine whether female tree swallows actually assess potential extra-pair mates according to feather length. Obviously, future studies should attempt to manipulate flight feather length in male tree swallows early in the breeding season to assess the importance of this trait for extra-pair mate choice by females. Logistic difficulties with capturing male tree swallows early in the breeding season, combined with a high potential for males to abandon a nest-site if captured early in the season (pers. obs.) may be prohibitive for such studies; however, if successful, this approach will allow us to determine both how female tree swallows assess potential mates, and what traits may be exposed to sexual selection via extra-pair fertilizations in this species.

Literature Cited

Alatalo RV, Lundberg A, Glynn C, 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature 323:152-153.

Andersson M, 1994. Sexual Selection. Princeton: Princeton University Press.

Ardia DR, 2005. Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. J Anim Ecol 74:517-524.

Arnqvist G, Kirkpatrick M, 2005. The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. Am Nat 165:S26-S37.

Ashton JC, Armstrong DP, 2002. Facultative prioritization of wing growth in the welcome swallow *Hirundo neoxena.* Ibis 144:470-477.

Barber CA, Robertson RJ, Boag PT, 1996. The high frequency of extra pair paternity in tree swallows is not an artifact of nestboxes. Behav Ecol Sociobiol 38:425-430.

Barber CA, Robertson RJ, Boag PT, 1998. Experimental mate replacement does not increase extra-pair paternity in tree swallows. Proc R Soc Lond 265:2187-2190.

Birkhead T, Schwabl H, Burke T, 2000. Testosterone and maternal effects – integrating mechanisms and function. Trends Ecol Evol 15:86-87.

Bize P, Roulin A, Bersier L-F, Pfluger D, Richner H, 2003. Parasitism and developmental plasticity in alpine swift nestlings. J Anim Ecol 72:633-639.

Bowlin MS, Winkler DW, 2004. Natural variation in flight performance is related to timing of breeding in tree swallows (*Tachycineta bicolor*) in New York. Auk 121:345-353.

Brown CR, Brown MB, 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. Evolution 52:1461-1475.

Brown CR, Brown MB, Rannala B, 1995. Ectoparasites reduce long-term survival of their avian host. Proc R Soc Lond B 262:313-319.

Buechler K, Fitze PS, Gottstein B, Jacot A, Richner H, 2002. Parasite-induced maternal response in a natural bird population. J Anim Ecol 71:247-252.

Burley N, 1986. Sexual selection for aesthetic traits in species with biparental care. Am Nat 127:415-445.

Burness GP, Ydenberg RC, Hochachka PW, 2001. Physiological and biochemical correlates of brood size and energy expenditure in tree swallows. J Exp Biol 204:1491-1501.

Byers BE, Mays HL Jr, Stewart IRK, Westneat DF, 2004. Extra-pair paternity increases variability in male reproductive success in the chestnut-sided warbler (*Dendroica pensylvanica*), a socially monogamous songbird. Auk 121:788-795.

Calsbeek R, Sinervo B, 2002. Uncoupling direct and indirect components of female choice in the wild. Proc Natl Acad Sci USA 99:14897-14902.

Charmantier A, Garant D, 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proc R Soc Lond 272:1415-1425.

Charmantier A, Blondel J, Perret P, Lambrechts MM, 2004. Do extra-pair paternities provide genetic benefits for female blue tits (*Parus caeruleus*)? J Avian Biol 35:524-532.

Christe P, Richner H, Oppliger A, 1996. Of great tits and fleas: sleep baby sleep.... Anim Behav 52:1087-1092.

Clotfelter ED, Whittingham LA, Dunn PO, 2000. Laying order, hatching synchrony, and nestling body mass in tree swallows (*Tachycineta bicolor*). J Avian Biol 31:329-334.

Conrad KF, Johnston PV, Crossman C, Kempenaers B, Robertson RJ, Wheelwright NT, Boag PT, 2001. High levels of extra-pair paternity in an isolated low-density, island population of tree swallows (*Tachycineta bicolor*). Mol Ecol 10:1301-1308.

Cotton MJ, 1970. The life history of the hen flea, *Ceratophyllus gallinae* (Schrank) (Siphonaptera, Ceratophyllidae). Entomologist 103:45-48.

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

Cunningham EJA, Russell AF, 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:74-77.

Dale S, Slagsvold T, 1996. Mate choice on multiple cues, decision rules and sampling strategies in female pied flycatchers. Behaviour 133:903-944.

Dale S, Rinden H, Slagsvold T, 1992. Competition for a mate restricts mate search of female pied flycatchers. Behav Ecol Sociobiol 30:165-176.

Darwin C, 1871. The descent of man and selection in relation to sex. New York: D. Appleton and Company.

Dawson RD, Lawrie CC, O'Brien EL, 2005. The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. Oecologia 144:499-507.

Dickinson JL, 2001. Extrapair copulations in western bluebirds (*Sialia mexicana*): female receptivity favors older males. Behav Ecol Sociobiol 50:423-429.

Dunn PO, Robertson RJ, Michaud-Freeman D, Boag PT, 1994. Extra-pair paternity in tree swallows: why do females mate with more than one male. Behav Ecol Sociobiol 35:273-281.

Fernando P, Evans BJ, Morales JC, Melnick DJ, 2001. Electrophoresis artifacts – a previously unrecognized cause of error in microsatellite analysis. Mol Ecol Notes 1:325-328.

Festa-Bianchet M, Coltman DW, Turelli L, Jorgenson JT, 2004. Relative allocation to horn and body growth in bighorn rams varies with resource availability. Behav Ecol 15:305-312.

Fitze PS, Tschirren B, Richner H, 2004. Life history and fitness consequences of ectoparasites. J Anim Ecol 73:216-226.

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B, 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714-717.

Forstmeier W, Kempenaers B, Meyer A, Leisler B, 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proc R Soc Lond 269:1479-1485.

Forstmeier W, Hasselquist D, Bensch S, Leisler B, 2006. Does song reflect age and viability? A comparison between two populations of the great reed warbler *Acrocephalus arundinaceus*. Behav Ecol Sociobiol 59:634-643.

Franchimont D, 2004. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. Ann NY Acad Sci 1024:124-137.

Garamszegi LZ, Heylen D, Møller AP, Eens M, de Lope F, 2005. Age-dependent health status and song characteristics in the barn swallow. Behav Ecol 16:580-591.

Gebhardt-Henrich SG, Richner H, 1998. Causes of growth variation and its consequences for fitness. In: Starck JM, Ricklefs RE (eds) Avian growth and development. Oxford University Press, Oxford, pp 324-339.

Gil D, Graves J, Hazon N, Wells A, 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286:126-128.

Gil D, Leboucher G, Lacroix A, Cue R, Kreutzer M, 2004. Female canaries produce eggs with greater amounts of testosterone when exposed to preferred male song. Horm Behav 45:64-70.

Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ, 1990. Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. Science 250:1394-1397.

Griffith SC, Owens IPF, Thuman KA, 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195-2212.

Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384-387.

Harper GH, Marchant A, Boddington DG, 1992. The ecology of the hen flea *Ceratophyllus gallinae* and the moorhen flea *Dasypsyllus gallinulae* in nestboxes. J Anim Ecol 61:317-327.

Hasselquist D, Bensch S, von Schantz T, 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. Nature 381:229-232.

Heeb P, Werner I., Kölliker M, Richner H, 1998. Benefits of induced host responses against an ectoparasite. Proc R Soc Lond B 265:51-56.

Hoffman AA, Merilä J, 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol Evol 14:96-101.

Holroyd GL, 1975. Nest-site availability as a factor limiting population size of swallows. Can Field Nat 89:60-64.

Houtman AM, 1992. Female zebra finches choose extra-pair copulations with genetically attractive males. Proc R Soc Lond 249:1324.

Humphries DA, 1968. The host-finding behaviour of the hen flea, *Ceratophyllus gallinae* (Schrank) (Siphonaptera). Parasitology 58:403-414.

Hussell DJT, 1983. Age and plumage color in female tree swallows. J Field Ornithol 54:312-318.

Jamieson A, 1994. The effectiveness of using codominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. Anim Genet 25:19-23.

Jeffreys AJ, Allen MJ, Hagelberg E, Sonnberg A, 1992. Identification of the remains of Mengele, Josef by DNA analysis. Forensic Sci Int 56:65-76.

Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev 72:283-327.

Jennions MD, Petrie M, 2000. Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21-64.

Jia F-Y, Greenfield MD, Colins RD, 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype x environment interaction. Evolution 54:953-967.

Johannessen LE, Slagsvold T, Hansen BT, Lifjeld JT, 2005. Manipulation of male quality in wild tits: effects on paternity loss. Behav Ecol 16:747-754.

Johnsen A, Andersen V, Sundling C, Lifjeld JT, 2000. Female bluethroats enhance offspring immunocompetence through extra-pair copulations. Nature 406:296-299.

Johnsen A, Lifjeld JT, Andersson S, Ornborg J, Amundsen T, 2001. Male characteristics and fertilization success in bluethroats. Behaviour 138:1371-1390.

Johnson LS, Albrecht DJ, 1993. Effects of haematophagous ectoparasites on nestling house wrens, *Troglodytes aedon*: who pays the cost of parasitism? Oikos 66:255-262.

Kempenaers B, Verheyen GR, Dhondt AA, 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring performance. Behav Ecol 8:481-492.

Kempenaers B, Congdon B, Boag P, Robertson RJ, 1999. Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? Behav Ecol 10:304-311.

Kempenaers B, Everding S, Bishop C, Boag P, Robertson RJ, 2001. Extra-pair paternity and the reproductive role of male floaters in the tree swallow (*Tachycineta bicolor*). Behav Ecol Sociobiol 49:251-259.

Kleven O, Lifjeld JT, 2004. Extrapair paternity and offspring immunocompetence in the reed bunting, *Emberiza schoeniclus*. Anim Behav 68:283-289.

Kleven O, Marthinsen G, Lifjeld JT, 2005. Male extraterritorial forays, age and paternity in the socially monogamous reed bunting (*Emberiza schoeniclus*). J Ornithol: published online Dec6, 2005; DOI 10.1007/s10336-005-0039-z.

Kleven O, Jacobsen F, Izadnegahdar R, Robertson RJ, Lifjeld JT, 2006. Male tail streamer length predicts fertilization success in the North American barn swallow (*Hirundo rustica erythrogaster*). Behav Ecol Sociobiol 59:412-418.

Leonard ML, Horn AG, 1996. Provisioning rules in tree swallows. Behav Ecol Sociobiol 38:341-347.

Leffelaar D, Robertson RJ, 1985. Nest usurpation and female competition for breeding opportunities by tree swallows. Wilson Bull 97:221-224.

Li P, Martin TE, 1991. Nest-site selection and nesting success of cavity-nesting birds in high elevation forest drainages. Auk 108:405-418.

Li S-H, Brown JL, 2002. Reduction of maternal care: a new benefit of multiple mating? Behav Ecol 13:87-93.

Lifjeld JT, Robertson RJ, 1992. Female control of extra-pair fertilization in tree swallows. Behav Ecol Sociobiol 31:89-96.

Lifjeld JT, Dunn PO, Robertson RJ, Boag PT, 1993. Extra-pair paternity in monogamous tree swallows. Anim Behav 45:213-229.

Ligon JD, 1999. The Evolution of Avian Breeding Systems. Oxford University Press, Oxford.

Limbourg T, Mateman AC, Andersson S, Lessells CM, 2004. Female blue tits adjust parental effort to manipulated male UV attractiveness. Proc R Soc Lond 271:1903-1908.

Lubjuhn T, Stronbach S, Brün J, Gerken T, Epplen JT, 1999. Extra-pair paternity in great tits (*Parus major*): a long term study. Behaviour 136:1157-1172.

MacDougall-Shackleton EA, Robertson RJ, Boag PT, 1996. Temporary male removal increases extra-pair paternity in eastern bluebirds. Anim Behav 52:1177-1183.

Magrath MJL, Green DJ, Komdeur J, 2002. Sex allocation in the sexually monomorphic fairy martin. J Avian Biol 33:260-268.

Malausa T, Guillemaud T, Lapchin L, 2005. Combining genetic variation and phenotypic plasticity in tradeoff modelling. Oikos 110:330-338.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-655.

Martins TLF, 1997. Fledging in the common swift, *Apus apus*: weight-watching with a difference. Anim Behav 54:99-108.

Mays HL, Hill GE, 2004. Choosing mates: good genes versus genes that are a good fit. Trends Ecol Evol 19:554-559.

McCarty JP, 2001. Variation in growth of nestling tree swallows across multiple temporal and spatial scales. Auk 118:176-190.

McCarty JP, 2002. The number of visits to the nest by parents is an accurate measure of food delivered to nestlings in Tree Swallows. J Field Ornithol 73:9-14.

Merino S, Potti J, 1996. Weather dependent effects of nest ectoparasites on their bird hosts. Ecography 19:107-113.

Merilä J, 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. Evolution 51:526-536.

Michaud T, Leonard M, 2000. The role of development, parental behavior, and nestmate competition in fledging of nestling tree swallows. Auk 117:996-1002.

Møller AP, 1992. Frequency of female copulations with multiple males and sexual selection. Am Nat 139:1089-1101.

Møller AP, 1994. Male ornament size as a reliable cue to enhance offspring viability in the barn swallow. Proc Nat Acad Sci USA 91:6929-6932.

Møller AP, Birkhead T, 1994. The evolution of plumage brightness in birds is related to extrapair paternity. Evolution 48:1089-1100.

Moss WW, Camin JH, 1970. Nest parasitism, productivity, and clutch size in purple martins. Science 168:1000-1003.

Neff BD, Pitcher TE, 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol Ecol 14:19-38.

Nilsson SG, 1984. The evolution of nest-site selection among hole-nesting birds: The importance of nest predation and competition. Ornis Scand 15:167-175.

Nilsson J-Å, 2003. Ectoparasitism in marsh tits: costs and functional explanations. Behav Ecol 14:175-181.

Norušis MJ, 2000. SPSS 11.0 Guide to data analysis. Upper Saddle River: Prentice Hall.

O'Brien EL, Morrison BL, Johnson LS, 2001. Assessing the effects of haematophagous ectoparasites on the health of nestling birds: haematocrit vs haemoglobin levels in House Wrens parasitized by blowfly larvae. J Avian Biol 32:73-76.

Oppliger A, Richner H, Christe P, 1994. Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit. Behav Ecol 5:130-134.

Otter K, Ratcliffe L, 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. Proc R Soc Lond 263:351-354.

Otter K, Ratcliffe L, Michaud D, Boag PT, 1998. Do female black-capped chickadees prefer high-ranking males as extra-pair partners? Behav Ecol Sociobiol 43:25-36.

Parker TH, 2003. Genetic benefits of mate choice separated from differential maternal investment in red junglefowl *(Gallus gallus).* Evolution 57:2157-2165.

Petrie M, Kempenaers B, 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends Ecol Evol 13:52-58.

Price T, Kirkpatrick M, Arnold SJ, 1988. Directional selection and the evolution of breeding date in birds. Science 240:798-799.

Primmer CR, Møller AP, Ellegren H, 1995. Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica.* Mol Ecol 4:493-498.

Primmer CR, Møller AP, Ellegren H, 1996. New microsatellites from the pied flycatcher *Ficedula hypoleuca* and the swallow *Hirundo rustica* genomes. Hereditas 124:281-283.

Qvarnström A, 1999. Genotype-by-environment interactions in the determination of the size of a secondary sexual character in the collared flycatcher (*Ficedula albicollis*). Evolution 53:1564-1572.

Qvarnström A, 2001. Context-dependent genetic benefits from mate choice. Trends Ecol Evol 16:5-7.

Rendell WB, 1993. Intraspecific killing observed in tree swallows, *Tachycineta bicolor*. Can Field Nat 107:227-228.

Rendell WB, Robertson RJ, 1989. Nest-site characteristics, reproductive success and cavity availability for tree swallows breeding in natural cavities. Condor 91:875-885.

Richner H, Heeb P, 1995. Are clutch and brood size patterns in birds shaped by ectoparasites? Oikos 73:435-441.

Richner H, Tripet F, 1999. Ectoparasitism and the trade-off between current and future reproduction. Oikos 86:535-538.

Richner H, Oppliger A, Christe P, 1993. Effect of an ectoparasite on reproduction in great tits. J Anim Ecol 62:703-710.

Rintamäki PT, Lundberg A, Alatalo RV, Höglund J, 1998. Assortative mating and female clutch investment in black grouse. Anim Behav 56:1399-1403.

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 (*Tachycineta bicolor*). In: Poole A, Gill F (eds) The Birds of North America, No.11. Philadelphia, PA: The Birds of North America Inc.

Ryan PG, 2001. Morphological heritability in a hybrid bunting complex: *Nesospiza* at Inaccessible Island. Condor 103:429-438.

Saino N, Primmer CR, Ellegren H, Møller AP, 1997. An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). Evolution 51:562-570.

Saino N, Incagli M, Martinelli R, Ambrosini R, Møller AP, 2001. Immunity, growth and begging behavior of nestling barn swallows *Hirundo rustica* in relation to hatching order. J Avian Biol 32:263-270.

Saino N, Ferrari RP, Romano M, Ambrosini R, Møller AP, 2002. Ectoparasites and reproductive trade-offs in the barn swallow (*Hirundo rustica*). Oecologia 133:139-145.

Saino N, Suffritti C, Martinelli R, Rubolini D, Møller AP, 2003. Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). Behav Ecol 14:318-325.

von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H, 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proc R Soc Lond 266:1-12.

Schmoll T, Dietrich V, Winkell 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.

Searcy WA, 1982. The evolutionary effects of mate selection. Ann Rev Ecol Syst 13:57-85.

Seutin G, White BN, Boag PT, 1991. Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82-90.

Sheldon BC, 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proc R Soc Lond 257:25-30.

Sheldon BC, 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol Evol 15:397-402.

Sheldon BC, Ellegren H, 1999. Sexual selection resulting from extrapair paternity in collared flycatchers. Anim Behav 57:285-298.

Sheldon BC, Merilä J, Qvarnström A, Gustafsson L, Ellegren H, 1997. Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. Proc R Soc Lond B 264:297-302.

Sheldon BC, Arponen H, Laurila A, Crochet P-A, Merilä J, 2003. Sire coloration influences offspring survival under predation risk in the moorfrog. J Evol Biol 16:1288-1295.

Shutler D, Clark RG, 2003. Causes and consequences of tree swallow (*Tachycineta bicolor*) dispersal in Saskatchewan. Auk 120:619-631.

Shutler D, Mullie A, Clark RG, 2004. Tree swallow reproductive investment, stress, and parasites. Can J Zool 82:442-448.

Siitari H, Honkavaara J, Huhta E, Viitala J, 2002. Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. Anim Behav 63: 97-102.

Slagsvold T, 1986. Nest-site settlement by the pied flycatcher: does the female choose her mate for the quality of his house or himself? Ornis Scand 17:210-220.

Slagsvold T, Drevon T, 1999. Female pied flycatchers trade between male quality and mating status in mate choice. Proc R Soc Lond 266:917-921.

Slagsvold T, Lifjeld JT, 1988. Ultimate adjustment of clutch size to parental feeding capacity in a passerine bird. Ecology 69:1918-1922.

Slagsvold T, Lifjeld JT, 1990. Influence of male and female quality on clutch size in tits (*Parus* spp). Ecology 71:1258-1266.

Slagsvold T, Lifjeld JT, Stenmark G, Breiehagen T, 1988. On the cost of searching for a mate in female pied flycatchers *Ficedula hypoleuca.* Anim Behav 36:433-442.

Smits JE, Bortolotti GR, Tella JL, 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. Funct Ecol 13:567-572.

Stapleton MK, 2005. Extrapair mating in tree swallows: an examination of the genetic compatibility hypothesis. PhD Dissertation, Queen's University, Kingston, Ontario, Canada.

Stutchbury BJ, Robertson RJ, 1988. Within-season and age-related patterns of reproductive performance in female tree swallows (*Tachycineta bicolor*). Can J Zool 66:827-834.

Stutchbury BJM, Piper WH, Neudorf DL, Tarof SA, Rhymer JM, Fuller G, Fleischer RC, 1997. Correlates of extra-pair fertilization success in hooded warblers. Behav Ecol Sociobiol 40:119-126.

Sætre GP, Svein D, Slagsvold T, 1994. Female pied flycatchers prefer brightly coloured males. Anim Behav 48:1407-1416.

Sætre G-P, Fossnes T, Slagsvold T, 1995. Food provisioning in the pied flycatcher: do females gain direct benefits from choosing bright-coloured males? J Anim Ecol 64:21-30.

Thomas K, Shutler D, 2001. Ectoparasites, nestling growth, parental feeding rates, and begging intensity of tree swallows. Can J Zool 79:346-353.

Tinbergen J M, Boerlijst MC, 1990. Nestling weight and survival in individual great tits (*Parus major*). J Anim Ecol 59:1113-1127.

Tripet F, Richner H, 1997. Host responses to ectoparasites: food compensation by parent blue tits. Oikos 78:557-561.

Tschirren B, Richner H, Schwabl H, 2004. Ectoparasite-modulated deposition of maternal androgens in great tit eggs. Proc R Soc Lond B 271:1371-1375.

Uller T, Eklöf J, Andersson S, 2005. Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. Behav Ecol Sociobiol 57:584-590.

Wagner RH, Schug MD, Morton ES, 1996. Condition-dependent control of paternity by female purple martins: implications for coloniality. Behav Ecol Sociobiol 38:379-389.

Webster MS, Pruett-Jones S, Westneat DF, Arnold SJ, 1995. Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution 49:1147-1157.

Webster MS, Chuang-Dobbs HC, Holmes RT, 2001. Microsatellite identification of extrapair sires in a socially monogamous warbler. Behav Ecol 12:439-446.

Weatherhead PJ, Boag PT, 1995. Pair and extra-pair mating success relative to male quality in red-winged blackbirds. Behav Ecol Sociobiol 37:81-91.

Welch AM, 2003. Genetic benefits of a female mating preference in grey tree frogs are context-dependent. Evolution 57:883-893.

Whittingham LA, Dunn PO, 2001. Survival of extrapair and within-pair young in tree swallows. Behav Ecol 12:496-500.

Whittingham LA, Dunn PO, 2005. Effects of extra-pair and within-pair reproductive success on the opportunity for selection in birds. Behav Ecol 16:138-144.

Whittingham LA, Dunn PO, Clotfelter ED, 2003. Parental allocation of food to nestling tree swallows: the influence of nestling behaviour, sex and paternity. Anim Behav 65:1203-1210.

Yasui Y, 1998. The ‘genetic benefits’ of female multiple mating reconsidered. Trends Ecol Evol 13:246-250.

Yezerinac SM, Weatherhead PJ, Boag PT, 1995. Extra-pair paternity and the opportunity for sexual selection in a socially monogamous bird (*Dendroica petechia*). Behav Ecol Sociobiol 37:179-188.

Appendix I

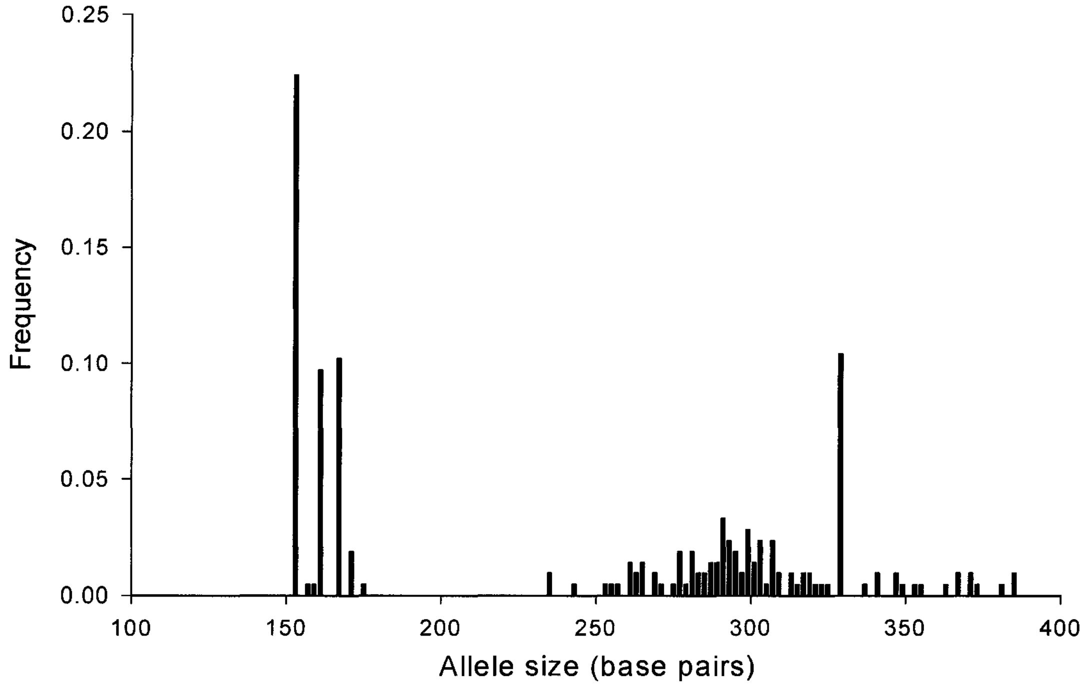

Fig. A.1 Allele sizes and frequencies for microsatellite HrU6, derived from a sample of 108 adult tree swallows.

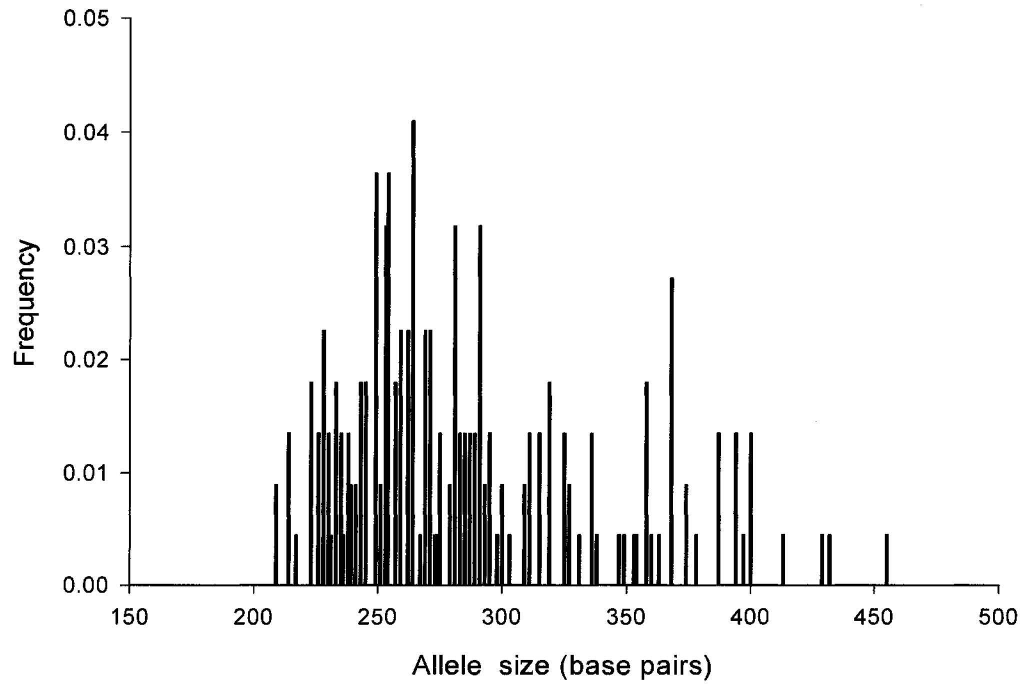

Fig. A.2 Allele sizes and frequencies for microsatellite HrU10, derived from a sample of 111 adult tree swallows.

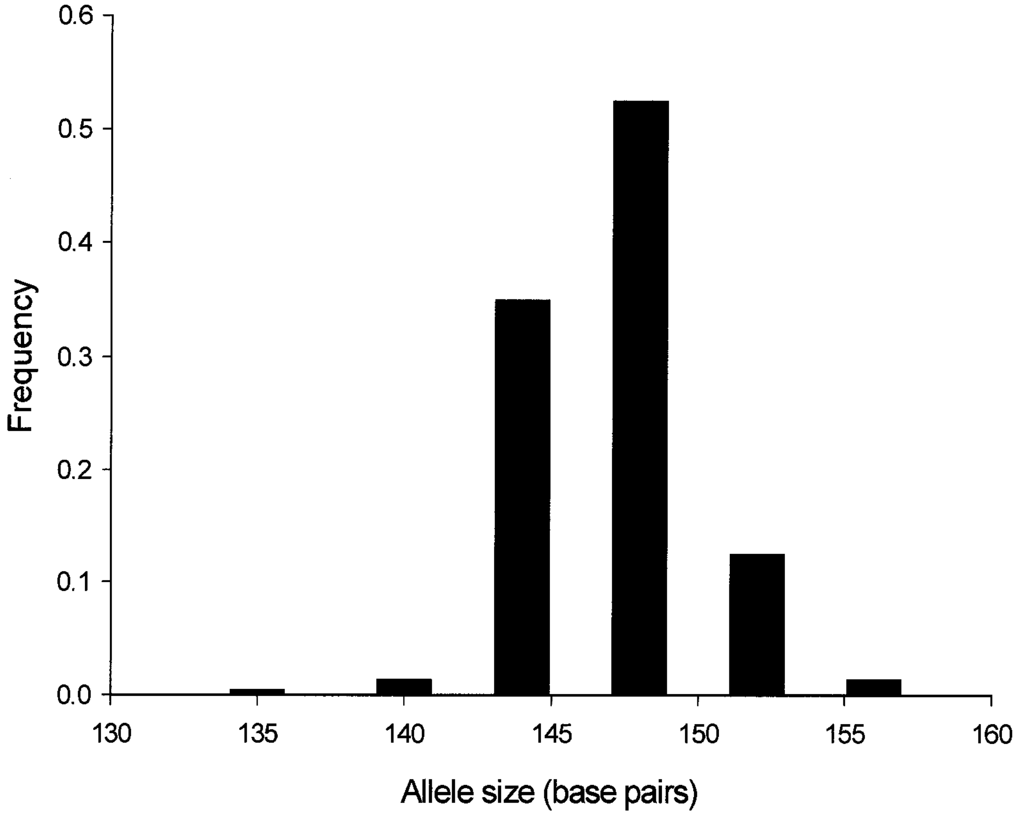

Fig. A.3 Allele sizes and frequencies for microsatellite IBI MP5-29, derived from a sample of 111 adult tree swallows.

Table A1. Summary of paternity information for nests of tree swallows breeding during 2004. Nests are numbered according to their relative geographic locations within the study area; thus, numbers that are closer together (e.g. 1 and 2) represent nests that were in closer proximity to each other than numbers further apart (e.g. 1 and 5). "WPO" and "EPO" are within-pair and extra-pair offspring, respectively. Extra-pair sires (EP sires) are identified by the number of the nest at which they were the resident male, followed, in parentheses, by the number of EPO they sired. "U" indicates that no EP sires were identified. Details of the chance inclusion probability calculation are presented in Chapter 3 (3.3.2).

Nest	Brood size	Offspring genotyped	WPO	EPO	EP sire(s)	Probability of chance inclusion
1	6	6	3	3	U	
2	5	5	4	1	U	
3	6	5	3	2	2 (2)	0.0006 0.0006
4	7	7	5	2	5 (2)	0.0049 0.0008
5	6	6	1	5	U	
6	7	7	7	0	-	
7	6	6	5	1	U	
8	7	7	1	6	1 (1) 5 (2) 14 (2) U (1)	0.0008 0.0008 0.0118 0.0023 0.0088
9	6	5	4	1	8 (1)	0.0004
10	6	5	3	2	U	
11	7	7	7	0	-	
12	4	3	3	0	-	
13	5	5	3	2	U	
14	6	5	5	0	-	
15	5	5	3	2	U	
16	2	2	2	0	-	
17	6	6	5	1	U	
18	4	4	3	1	U	
19	4	4	1	3	20 (3)	0.0023 0.0023 0.0088
20	3	4	3	1	U	
21	5	5	3	2	22 (1) 27 (1)	0.0242 0.0013

Nest	Brood size	Offspring genotyped	WPO	EPO	EP sire(s)	Probability of chance inclusion
24	6	6	3	3	19 (2) 23 (1)	0.0009 0.0004 0.0001
25	6	6	3	3	32 (3)	0.0053 0.0006 0.0088
26	7	7	6	1	25 (1)	0.0003
27	7	7	5	2	U	
28	4	4	1	3	U	
29	6	5	1	4	21 (1) 23 (2) 37 (1)	0.0039 0.0002 0.0003 0.0027
30	6	6	5	1	U	
31	6	6	3	3	U	
32	6	5	2	3	38 (1) 43 (1) U (1)	0.0011 0.0006
33	4	4	1	3	U	
34	7	7	1	6	U	
35	7	7	6	1	U	
36	6	6	4	2	39 (2)	0.0006 0.0005
37	6	6	6	0	-	
38	5	5	4	1	U	
40	7	7	5	2	U	
41	6	6	5	1	40 (1)	0.0075
42	6	5	2	3	29 (1) U (2)	0.0138
43	6	4	3	1	U	