VARIATION IN EXTRA-PAIR MATING SYSTEMS IN *TACHYCINETA* SWALLOWS: A LIFE-HISTORY APPROACH

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VARIATION IN EXTRA-PAIR MATING SYSTEMS IN *TACHYCINETA* SWALLOWS: A LIFE-HISTORY APPROACH

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Variation in life-history traits has long captivated ecologists and evolutionary biologists. Early contributors identified latitudinal clines in life-history traits and proposed ecological hypotheses to explain this variation. One ecological hypothesis proposed to explain geographic variation in extra-pair paternity (EPP) is the breeding synchrony hypothesis. Under this hypothesis, synchronously breeding females will be better able to assess the quality of potential mates when making mating decisions. The prediction in this hypothesis is that synchrony increases towards the poles because of shorter breeding seasons; rates of EPP are therefore expected to increase towards higher latitudes. Simultaneously, recent comparative work found that most diversification in avian EPP occurred early in the evolutionary history of birds, with most variation found between Families and Orders. In my dissertation I explore these two perspectives by examining interspecific variation in genetic mating system in the swallow genus *Tachycineta*. I obtained EPP data using microsatellite markers for five species of *Tachycineta* swallows ranging from Tierra del Fuego to British Columbia. *Tachycineta* swallows exhibit substantial variation in EPP, with 12 to 89% of nests having extra-pair young. A notable example of this variation is found between the sister taxa *T. leucorrhoa* and *T. meyeni*, with 78% and 12% of nests with extra-pair young respectively. My results indicate that breeding synchrony is not a strong predictor of EPP rates across species and latitudes. Additionally, I provide a detailed analysis of fitness benefits of EPP for a south-temperate species, *T. leucorrhoa*. I found that *T. leucorrhoa* nests with extra-pair young fledge more offspring compared

to those with all within-pair young. However, I did not find support for a link between this fitness advantage and the level of heterozygosity as proposed by theory. Work on *Tachycineta* helps redress the paucity of information on tropical and south-temperate species and an underrepresentation of closely related taxa that characterized previous studies. It also reminds us of the complexity of relationships among life-history traits and their environmental influences, forcing us to consider more than one hypothesis and causal path in explaining hemisphere-wide patterns in life histories.

BIOGRAPHICAL SKETCH

In 1994 Valentina joined the Laboratory on Animal Behavior at the IBYME (Institute of Biological and Experimental Medicine) in Buenos Aires, Argentina. She was an undergraduate at the University of Buenos Aires, and she did not imagine that a few years down the road she would end up migrating north to study birds. At the IBYME she started working under the supervision of Prof. Fabián Gabelli, who was studying the effects of ecological variations on the song dialects of Sedge Wrens. During extensive talks and field trips with Fabián, Valentina became interested in behavioral variation and life-history evolution, and she was soon drawn to studying the patterns of variation in the breeding biology of birds and their adaptations to very different ecological situations.

From 1997 to 2000 Valentina worked with Dr. Thomas Martin's field crew from the University of Montana. She spent two seasons in the United States (both at the Arizona field site and at the University of Montana); two seasons in Argentina (Parque Nacional El Rey); and one season supervising a field crew of biologists in the Ecuadorian cloud forest. Dr. Martin became an important guide and teacher to her and her scientific thinking, someone who challenged the conventional views of life-history theory.

As an undergraduate at the University of Buenos Aires, Valentina worked on her undergraduate thesis under the supervision of Dr. Juan Carlos Reboreda, starting in 1998 and finishing in 2000. The topic was parental care of the Rufous-bellied Thrush, and the thesis was approved with honors. The research with both Dr. Martin

and Dr. Reboreda helped her further develop her interests on the evolution of parental care and mating systems from a life-history perspective.

In 2002 Valentina was accepted to the Graduate Program in the Department of Ecology and Evolutionary Biology at Cornell University, under the supervision of Dr. David Winkler and Dr. Irby Lovette. In 2004 she completed her M.S. on the costs of polygyny in the Tree Swallow, and from then on she focused on the doctoral research that is part of this dissertation.

During her stay in Ithaca and her studies at Cornell University, there were several ups and downs. She can say, however, that the downs were cushioned with friendship—Valentina met some of her best friends during this time. She was also lucky to have two wonderful supervisors covering her back. She learned many things from her advisors but, above all, the difference between being a supervisor and being a mentor. Both Dr. Winkler and Dr. Lovette played a major role in her development as a researcher and in the way she approaches science today. Although very different in their personalities, they have both inspired Valentina with their passion for biology and their quest for answers. She is leaving Cornell knowing that she has in Ithaca two great mentors and friends.

At a Ph.D. recognition ceremony at Cornell, Valentina once heard a professor say that earning a Ph.D. is more than anything a test of character. It is the best description she has ever heard of graduate school.

Our greatest glory is not in never falling, but in rising every time we fall. – Confucius

In loving memory of my father Ezio Ferretti and to my mother Franca N. de Ferretti, both of whom taught me how to rise after a fall

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CHAPTER 1

BREEDING DENSITY, FEMALE BREEDING SYNCHRONY AND EXTREME RATES OF EXTRA-PAIR PATERNITY IN A SOUTH TEMPERATE BIRD, THE WHITE-RUMPED SWALLOW *TACHYCINETA LEUCORRHOA*

Abstract

Despite the fact that the majority of bird species inhabit the tropics and southern hemisphere, studies of north temperate birds have historically driven much of the development of avian behavioral ecology and life history evolution. Here we characterize the genetic mating system of a south temperate breeder, the White-rumped Swallow *Tachycineta leucorrhoa*, and examine the effects of breeding density and synchrony on extra-pair paternity in this species. White-rumped Swallows nesting in Buenos Aires Province, Argentina, show notably high rates of extra-pair paternity, with 77% of nests (N = 78) having extra-pair young and 56% of nestlings (N = 342) in the sampled population being extra-pair. Within broods, one to four males fathered extra-pair offspring, and in 29% of nests all offspring were from extra-pair sires. In those cases where we could identify extra-pair sires (N = 31 males), the median distance between their own social nests and the nests where they fathered nestlings was 165m. We did not find a relationship between breeding synchrony and extra-pair paternity rates, nor between density of neighbors and extra-pair paternity. Previous comparative studies on life history traits have found most variation in extra-pair paternity to be concentrated at the taxonomic levels of family and order. Extra-pair paternity rates in our White-rumped Swallow populations are similar to those found in the north temperate congener, Tree Swallow *T. bicolor* (73-89% nests, 35-69% young), but quite different from those of their tropical relative, Mangrove Swallow *T.*

albilinea (26% nests, 15% nestlings), highlighting the need for more comprehensive sampling of species in this group to fully characterize interspecific variation in rates of extra-pair mating.

Introduction

Until the mid-1980s, most ecological research on avian mating systems focused on understanding variation in the number of an individual's social partners during each breeding attempt, and across its lifetime. However, since the earliest applications of molecular paternity-assessment techniques to avian systems (Burke and Bruford 1987), researchers have grown increasingly interested in variation in the number of genetic mates. It is now widely accepted that true genetic monogamy occurs in a minority of bird species—for example 14% of the passerine species included in a survey by Griffith et al. (2002). In contrast, more than 90% of all avian taxa are socially monogamous (Lack 1968). Underlying these broad trends, however, is considerable variation in the extent and prevalence of extra-pair paternity (EPP) rates within and between species (Griffith et al. 2002). The proximate and evolutionary drivers of this notably high variation in avian genetic mating systems remains an important, but still poorly understood, aspect of avian behavioral ecology (Arnold and Owens 2002, Bennett and Owens 2002).

Comparative analyses have found variation in life history traits to have a phylogenetic component (Owens and Bennett 1995, Bennett and Owens 2002). These analyses suggest that much of the variance in EPP rates arose early in the evolutionary history of birds, with 55% of this variation found at the deep level of families and orders (Arnold and Owens 2002). Thus, the pattern of variation in genetic mating systems observed at present may be due to current or recent selection factors

interacting with lineage-specific ancient predispositions towards high or low rates of EPP (Bennett and Owens 2002). Current ecological factors, like those affecting the likelihood of encountering potential extra-pair partners, either temporally or spatially—breeding density (Møller and Birkhead 1993) and local breeding synchrony (Stutchbury and Morton 1995)—are likely to interact with the evolutionary predisposition of any particular taxon to having high rates of EPP (Bennett and Owens 2002). Both temporal and spatial distribution of mates have long been suggested to influence mating systems (Emlen and Oring 1977), but the expected effects of these ecological factors on paternity are still controversial (Bennett and Owens 2002, Griffith et al. 2002). Our ability to tease apart these influences from those resulting from phylogenetic inertia is limited by the still sparse sampling of EPP in birds overall, and the rarity of samples from multiple closely allied taxa. In particular, even when closely related bird species are found across a broad latitudinal range, for the most part we lack information on EPP and other life history traits from their tropical and southern hemisphere representatives, which could be experiencing very different ecological conditions (Martin 2004, Neudorf 2004, Macedo et al. 2008, Stutchbury and Morton 2008).

Tree Swallows (*Tachycineta bicolor*) have become a model system for research in avian behavioral ecology, including the study of social and genetic mating biology (Jones 2003), and at least 34 papers have investigated patterns of EPP in this species. Tree Swallows have one of the highest rates of EPP among birds, and probably the highest known rate of any socially monogamous passerine, with 73-89% of nests having at least one extra-pair young, and 35-69% of nestlings being extra-pair (e.g., Dunn et al. 1994, Barber et al. 1996, Kempenaers et al. 1999, Whittingham and Dunn 2001, Whittingham et al. 2006, O'Brien and Dawson 2007, Stapleton et al.

2007, Crowe et al. 2009, Dunn et al. 2009). This species breeds exclusively at north temperate latitudes (Robertson et al. 1992), but eight other *Tachycineta* swallow species are found throughout the Americas (Turner and Rose 1989). Genetic mating patterns have been documented for only one of these additional species, the Mangrove Swallow (*T. albilinea*). Rates of EPP in this tropical breeding species are substantially lower than in Tree Swallows, with 26% nests containing at least one extra-pair young and 15% of the nestlings overall being sired by extra-pair males (Moore et al. 1999).

As exemplified by the differing EPP rates between congeneric Tree and Mangrove Swallows, there remains considerable variation in EPP at lower taxonomic levels of recent divergence. Here, we extend the study of genetic mating system variation in *Tachycineta* to a south temperate breeder, by providing the first detailed information on EPP rates for the White-rumped Swallow (*T. leucorrhoa*). In addition, we use this species to test for a positive relationship between EPP and increased opportunities for finding extra-pair mates by exploring the effects of nest density and breeding synchrony on paternity (Møller and Birkhead 1993, Stutchbury and Morton 1995).

Materials and methods

Field methods

Tachycineta swallows are secondary cavity nesters and most species readily breed in artificial nest boxes placed in study colonies. The White-rumped Swallow's breeding distribution ranges from Buenos Aires Province (Argentina) in the south to northern Bolivia and southern Brazil in the north (Turner and Rose 1989). Our work was conducted at a breeding colony in Chascomús, Buenos Aires (35º34'S, 58º01'W), where 126 nest boxes were spaced at 25-35m distances. For each box we recorded

latitude and longitude with an accuracy of < 3m using a Garmin 76 GPS. Swallows were studied for two consecutive years (2006-2007), from the start of the breeding season in September until early January.

White-rumped Swallows are socially monogamous, and both males and females contribute to the care of the young during the breeding season (Bulit et al. 2008). At our colony some pairs raise two broods within a breeding season (Massoni et al. 2007), but double-brooding is absent or rare (< 2%) at nearby colonies at similar latitudes (VF unpubl data). To assess the fate of nests, boxes were checked every other day from egg laying until nestlings were 15 days old. For each breeding attempt we recorded lay date (i.e. date of the first-laid egg), clutch size, and brood size. Clutches were considered complete when their size did not change for at least two days.

Genetic sample collection

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

before they were banded and bled, we collected a sample from their pectoral muscle and stored it in 96% ethanol.

Microsatellite amplification for paternity exclusions and assignments

We extracted DNA from blood and muscle samples using DNA purification kits by Eppendorf and Qiagen. Extracted DNA was diluted 1:10 in ultrapurified H_2O and then amplified at 12 highly polymorphic microsatellite loci (Table 1.1) following the conditions from Makarewich et al. (2009). We amplified multiple loci in multiplexed polymerase chain reactions (PCR); this allowed us to score all 12 loci with only three PCR reactions per individual. The combination of primers used in each of these multiplexed reactions was selected so as to avoid PCR product overlap by their fragment sizes as well as by using unique fluorescent dyes. PCRs were performed in 10µl final volumes. Each of the three mixes used 10-100ng DNA, 10mM Tris-HCl (ph 8.3), 50mM KCl, 3.25mM $MgCl_2$, 200µM dNTPs (Invitrogen), 0.25U Jumpstart *Taq* polymerase (Sigma), the specified mix of forward and reverse primers, and H_2O to bring the final volume to 10µl. Mix 1 contained 1pM Tle19, 2.4pM Tle17, 1pM Tle16, 4.8pM Tabi4 and 1.2pM Tabi1 forward and reverse primers. Mix 2 contained 2.4pM Tle4, 1.2pM Tle8 and 2.4pM Tal7 forward and reverse primers. Mix 3 contained 1.2pM Tle21, 1.8pM Tal11, 3.6pM Tal8 and 1.6pM Tal6 forward and reverse primers.

PCRs were performed in a DYAD thermal cycler (Bio-Rad). Cycling profiles for mixes 1 and 2 followed one incubation cycle of 95ºC for 2min; 35 cycles of 50s at 95ºC, 1min at an annealing temperature of 56ºC, and an extension time of 1min at 72ºC; these 35 cycles were followed by a final extension phase of 30min at 72ºC. Mix 3 PCR cycle was the same as for 1 and 2 with the exception that the annealing temperature used was 58ºC. PCR products were then genotyped on an ABI PRISM

3100 Genetic Analyzer (Applied Biosystems), and the sizes of the microsatellite alleles estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems).

Table 1.1 Microsatellite loci used in this study and their characteristics. N: number of unrelated individuals genotyped, N_A: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, PCR Mix: primers used in the same multiplexed PCR reaction, equal numbers represent primers used in the same multiplex PCR reaction.

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

Allele frequencies and population genetic data were generated using the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Paternity

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

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

Measures of female breeding synchrony

We characterized each female's fertile period as spanning six days prior to the date the first egg was laid (Ardia et al. 2006) through the lay date of the penultimate egg (Moore et al. 1999). We calculated a female synchrony index using the following formula:

$$SI_p = \left[\frac{\Sigma_{i=1}^{t_p} f_{i,p}}{t_p (F-1)} \right] \cdot 100$$

with SI_p being the synchrony index for each female p in the population; $f_{i,p}$ the number of fertile females, excluding female p, in day i; F the number of total females breeding in the population; and t_p the number of fertile days for female p (Kempenaers 1993, Stutchbury et al. 1998). This index is a measure of the overlap of the fertile period of each female with respect to that of the rest of the breeding females in the population.

Measures of breeding density

We calculated distances between nest boxes, using their geographic coordinates, with the program Geographic Distance Matrix Generator (Ersts,P.J. [Internet] Geographic Distance Matrix Generator 1.2.3. American Museum of Natural History, Center for Biodiversity and Conservation. Available from http://biodiversityinformatics.amnh.org/open_source/gdmg). We did this for all active nest boxes in each year. For each focal active nest we counted the number of other nests that were active during the fertile period of the focal nest within a set radius, and used this number as our measure of density. By doing this we took into account only the density of active nests during the focal females' fertile periods, the temporal window during which females will be seeking extra-pair copulations. We repeated this procedure for radii of successive 100m intervals from 100 to 300m. Densities were calculated with an R script, available from DWW. In addition, for those cases in which we could identify the genetic father we measured the distance between that male's nest

box and the box where he fathered young and calculated the median box-distance for these cases.

Table 1.2 Pearson's correlation coefficients for synchrony and density for all single broods and first broods/attempts in the colony. SI_p: female synchrony index; density100, 200 and 300: number of active nests within a radius of 100 m, 200m and 300m, respectively. N: sample size reflects the total number of nests in our population for which we had exact lay date information, regardless of whether these nests were sampled for paternity analyses.

Variables	Correlation coefficients	Sig. (2-tailed)	N
SI_p and density100*	0.442	< 0.001	144
SI_p and density200*	0.619	< 0.001	144
SI_p and density300*	0.653	< 0.001	144

*: We expected breeding density to be correlated with breeding synchrony since our density estimations are based on nests that overlap in their breeding cycles—that is, only those nests in the specified radius that were active during the fertile period of the female in the focal nest were considered for our density measures.

Statistical analyses

We used a binary logistic regression to test for the ability of density and synchrony to explain EPP status (presence or absence of extra-pair young in any one nest). We knew *a priori* that some of these predictors would be correlated (i.e. density at 100m and density at 200m). We first confirmed these correlations (Table 1.2) and then ran different models with each of the predictor variables. We ended with four logistic regression models: one using synchrony index as the only predictive variable; and

three additional models, one for each of the measures of density as predictors. In all four logistic regressions we used only information from first broods within a season. Analyses were run on JMP 8.0.1 (SAS Institute Inc. 2009) and SPSS 14 (SPSS Inc. 2005).

Results

We captured a total of 171 adults (87 females and 84 males). Some pairs are more reluctant to get in the nest boxes when the traps are set up; hence we failed to catch a few adults in our nest-box population (both males and females). The percentage of males captured in the colony was 75%. As some of the pairs in our population raise two broods within a breeding season, we present two sets of results in Table 3: one summary that takes into account all the nests sampled, and one in which only first broods are considered. We studied 78 broods of 55 breeding pairs and genotyped 342 nestlings (Table 1.3). In addition, we genotyped 22 resident males that were captured in our study area.

We found the total rate of EPP in our population to be 77% for broods with extra-pair young and 56% for extra-pair nestlings (Table 1.3). Twenty-three of the extra-pair nests had all nestlings sired by extra-pair males (29% broods, Table 1.3). To confirm these were not misidentifications of the social male or laboratory sample switches, we first re-genotyped all individuals in these family groups; and then cross-checked our information with that of the video recordings of parents feeding nestlings used in a concurrent study on parental care. In all 23 cases we derived the same genotypes for the individuals in these families, and the male bringing food to the nestlings was the one with the markings added at the time of capture/sampling. Within this group we identified four nests (5% of the total broods sampled) for which the

social male, despite having lost all the paternity at his nest, sired offspring at neighboring nests. Other males in this group might have also sired offspring in nests that were not sampled by us.

Table 1.3 Summary of EPP rates for White-rumped Swallows.

	All nests	**First broods**
Total number of nests	78 nests	55 nests
Total number of nestlings	342 nestlings	246 nestlings
Nests with extra-pair offspring	60 nests (77%)	43 nests (78%)
Nests with all extra-pair offspring	23 nests (29%)*	17 nests (31%)*
Extra-pair offspring	193 nestlings (56%)	136 nestlings (55%)

*: The percentage of nests containing all extra-pair offspring is based on the total number of nests sampled in the population, not just those with extra-pair young.

Of the 342 nestlings sampled, 193 were extra-pair offspring (56%, Table 1.3). We were able to identify the biological father for 90 of the 193 extra-pair nestlings (46.63%) with high probability. Within broods, one to several males sired extra-pair young—from our assignments we were able to detect up to four extra-pair males, although there could have been more in the frequent cases where we could not assign all biological fathers of the extra-pair offspring. The median distance between the nest of the extra-pair males and the nests where they fathered young was 165m (mean ± std. error = 262.31 ± 39.59m, N = 31 males, Max = 798.36m, Min = 23.91m, Figure 1.1).

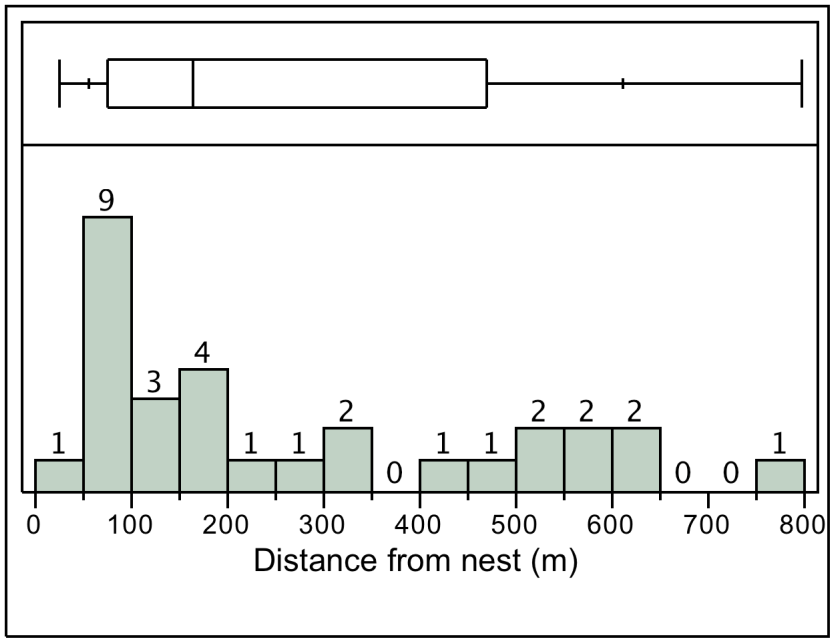

Figure 1.1 Histogram of distances between the extra-pair males' own nests and the nests where they sired offspring. Bin width is 50m. The numbers above the bars indicate the number of cases in each category. Box plot depicts the 25-75% quartiles of the distribution, with the line dividing the box at 50% being the median distance of 165m.

We did not find female breeding synchrony to be a significant predictor of EPP status (Table 1.4, model I; power for detecting between-group differences in mean synchrony was 0.71). Likewise, we found none of the density measures to be significant predictors of EPP (Table 1.4, models II, III and IV, power for density at 100m = 0.93, at 200m = 0.23, at 300m = 0.08).

Table 1.4 Logistic regression tests for the effects of different predictors on EPP status. I: female breeding synchrony index. II-IV: number of active nests within a radius of 100m, 200m and 300m, respectively. N: total number of single broods and first broods sampled for which we had exact lay date information, necessary for our calculations of breeding synchrony and density.

Model	Predictor variables	Chi-square	Prob>Chi-sq	N
I	Synchrony	1.14	0.285	52
II	Density100	1.99	0.158	52
III	Density200	0.29	0.592	52
IV	Density300	0.05	0.816	52

Discussion

Like most passerine birds, White-rumped Swallows engage in extra-pair mating. These swallows, however, are unusual in that their rate of EPP is far higher than that of most socially monogamous songbirds. We found that 77% of nests had extra-pair young and more than half of the total offspring (56%) were sired by extra-pair males (Table 1.3), whereas across other passerines the corresponding means are 18.7% of broods and 11.1% of offspring (Griffith et al. 2002). Adult White-rumped Swallows show a promiscuous behavior in that within broods, offspring are frequently sired by more than two males, and nearly 29% of the nests in our population had all nestlings sired by one to several extra-pair males (Table 1.3). The proportion of nests in the population where the socially attendant male lost all paternity appears higher than that observed in Tree Swallows (29% of nests versus 9.25-18.4% of nests in Tree Swallow, Kempenaers et al. 1999, Whittingham and Dunn 2001). However, a few of these males

that lost paternity at their own nests sired multiple offspring at neighboring boxes, suggesting a compensatory benefit.

Increased opportunities for mating have been suggested to be positively associated with EPP rates (Griffith et al. 2002, Neudorf 2004), both spatially—through a higher availability of mates in close proximity (Møller and Birkhead 1993)—and temporally—as females will be better able to assess the quality of potential extra-pair mates when breeding synchronously (Stutchbury and Morton 1995). We found, however, that neither breeding synchrony (Table 1.4, model I) nor nest density (Table 1.4, models II to IV) were good predictors of EPP status in this population of swallows. These ecological variables might be important for species in which males hold territories and compete to gain access to females, and in species that do not engage in long foraging/roosting trips during the female's fertile period. In contrast, the mating system of White-rumped Swallows may derive from a combination of intense pressure for securing nesting sites and their foraging ecology. White-rumped Swallows are aerial insectivores, and are similar to Tree Swallows in that they are quite aggressive at defending their nest boxes when other swallows approach them closely (Massoni et al. 2007). Both male and female White-rumped Swallows vigilantly guard their nest boxes when the other member of the pair is out foraging (VF pers obs). Once nests are secured, extra-pair copulations can be obtained by females anywhere within their foraging range—which, if similar to that of Tree Swallows, can extend from the nearest box neighbor to several kilometers when searching for food and roosting sites (Dunn and Whittingham 2005, 2007). A study on Tree Swallows found that prior to egg laying females move >2km to evening roosting sites and >10km when foraging during the day (Dunn and Whittingham 2005), although these distances might be affected by the condition of the females at the time

of breeding and the costs of searching for extra-pair mates for each female (Dunn and Whittingham 2007). In fact, an increased search cost of finding mates was found to affect the spatial distribution, but not the level, of extra-pair mating in Tree Swallows (Dunn and Whittingham 2007). In addition, a study conducted by Kempenaers et al. (1999) on the Tree Swallow, in which all the breeding males in the population were captured and sampled, found that only 21% of the extra-pair young were sired by resident males, suggesting that females obtain most of their extra-pair fertilizations outside localized concentrations of nesting sites. White-rumped Swallows in our nest-box population might show a similar mating pattern: the density of neighbors did not have a significant effect on EPP, suggesting that some of the females might be getting copulations beyond a 300m radius. However, it is likely that a smaller proportion of these females procure copulations beyond the limits of our colony, as we were able to assign a higher percentage of extra-pair offspring (46.63%) to one of the resident males in the local population, even when only 75% of these males were sampled (the median distance between the box of the extra-pair male and the box where he sired offspring was 165m, Figure 1.1). Consequently, EPP rate in these birds appears to arise from a variable mixing between opportunities for mating with local neighbors and more ephemeral pairings with more distant genetic mates encountered in foraging and roosting areas that may include birds from multiple breeding concentrations.

White-rumped Swallows, like their northern congener the Tree Swallow, have one of the highest rates of EPP in monogamous passerines (Table 1.3; for Tree Swallows: Dunn et al. 1994, Barber et al. 1996, Kempenaers et al. 1999, Whittingham and Dunn 2001, Whittingham et al. 2006, O'Brien and Dawson 2007, Stapleton et al. 2007, Crowe et al. 2009, Dunn et al. 2009); however, their EPP rates differ markedly from those in the tropical Mangrove Swallow (Moore et al. 1999). This difference

could well be a result of ancient diversification at the genus level, as suggested by Arnold and Owens (2002), favoring promiscuous behavior in *Tachycineta*; and of ecological facilitation currently selecting towards genetic monogamy in the tropical species. Testing this idea requires sampling of additional species within this monophyletic genus. Only then can we look at the integration of ecological and evolutionary factors in a phylogenetically constrained sample that will help us truly understand the current selective pressures influencing mating systems in birds.

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CHAPTER 2

FEMALE WHITE-RUMPED SWALLOWS (*TACHYCINETA LEUCORRHOA*) FLEDGE MORE YOUNG, BUT DO NOT INCREASE OFFSPRING HETEROZYGOSITY, THROUGH EXTRA-PAIR MATINGS

Abstract

There is growing evidence that females that engage in extra-pair matings choose males with whom they are more genetically compatible, increasing their offspring's fitness through increased heterozygosity. We looked at fitness benefits of extra-pair matings and tested the heterozygosity hypothesis in White-rumped Swallows (*Tachycineta leucorrhoa*) breeding in Argentina using a panel of 12 highly variable microsatellite loci. We found that broods with extra-pair offspring fledged more young than broods with no extra-pair offspring. Extra-pair offspring had a higher probability of surviving than within-pair offspring, but these two groups did not differ in their level of heterozygosity. Overall, young that died were more heterozygous than the ones that fledged. Neither the heterozygosity of the female nor her social mate, nor the genetic similarity of the social pair, predicted the presence of extra-pair young. Instead, females chose extra-pair mates that were more genetically similar to themselves. Our results do not support the heterozygosity hypothesis, suggesting that other mechanisms should be considered to explain the adaptive function of female choice for extra-pair mates.

Introduction

Since the first application of molecular techniques to studies of parentage in birds in the late 1980's (Burke and Bruford 1987) much progress has been made in our

understanding of avian mating systems and their variation. Matings outside the pair bond have been suggested to be advantageous for males by directly increasing the number of offspring sired in a given season (reviewed in Birkhead and Møller 1992). Fitness benefits of extra-pair mating for females, however, have been more difficult to identify due to the indirect nature of most such benefits (Jennions and Petrie 2000, Arnqvist and Kirkpatrick 2005, Akçay and Roughgarden 2007).

By engaging in extra-pair copulations (EPCs) females can modify their choice of a partner after securing a social mate, resulting in a mixed reproductive strategy. Petrie and Lipsitch (1994) suggested that, if it pays females to seek indirect genetic benefits through extra-pair matings, then females should mate with more than one male only when there is sufficient genetic variation among males. However, if there is a preference for a particular heritable male trait among females, the genetic variability on this trait in the population soon will be lost (Tregenza and Wedell 2000). An alternative explanation for female choice is based on the idea that there is no single best male for the female population, and that females will choose the male whose alleles best complement her own (i.e. most genetically compatible, Zeh and Zeh 1996, 1997, Brown 1997, Jennions 1997). This hypothesis suggests that selection on females will favor a choice of males such that their offspring will be heterozygous at some or several loci, which should result in increased offspring fitness ("heterozygosity theory," Brown 1997, Hansson and Westerberg 2002). Although this hypothesis remains controversial (Wetzel and Westneat 2009), the underlying assumption is that offspring resulting from genetically compatible matings are less likely to suffer the negative effects of inbreeding (Brown 1997).

In this paper we examine the effects of genetic compatibility, through microsatellite profiling, on offspring fitness and its relation to extra-pair paternity (EPP) in the White-rumped Swallow (*Tachycineta leucorrhoa*). White-rumped Swallows are good candidates for this analysis, because they are socially monogamous birds that show high levels of EPP (up to 77% of broods have at least one extra-pair young and 56% of nestlings in the population are extra-pair), and one brood can have multiple sires (Ferretti et al. Chapter 1).

Materials and methods

Field methods

We studied a population of White-rumped swallows breeding in nest-boxes in Buenos Aires province, Argentina (35º34'S, 58º01'W) during two breeding seasons (2006-2007). Details on the field site are given in Ferretti et al. (Chapter 1).

To assess the fate of nests, boxes were checked every other day from the start of egg laying until nestlings were 15 days old. For each breeding attempt we recorded lay date (i.e. date of the first-laid egg), clutch size, and brood size. We did not check nests with nestlings older than 15 days to avoid premature fledging of the young; we did, however, continue to monitor nests from a distance to ensure they remained active until fledging. The status of social mates was confirmed at each nest by observations that the social mate was the only male provisioning the focal nest. Once parental activity ceased at the nest—usually after day 20—we checked for dead nestlings inside the box and calculated the number of fledglings as the number of nestlings seen at the nest on day 15 minus the number of dead nestlings inside the box; after nestling day 10, parents generally do not remove dead chicks, and the resulting carcasses can later be found at the bottom of the nest cup once all the other chicks have fledged.

Nests were considered depredated if eggs or young disappeared when they were too young to fledge. Clutches were considered complete when their size did not change for at least two consecutive visits.

Adult females were captured during incubation and adult males were captured soon after the nestlings hatched. We captured adults at the nest-boxes using box traps described in http://golondrinas.cornell.edu. Adults and 8-10 day-old nestlings were banded with an aluminum band with a unique number. We collected a blood sample from all banded individuals by brachial venipuncture, and stored this sample in Queen's lysis buffer (Seutin et al. 1999).

Genetic analyses

Details on the protocols followed for paternity analyses have been described in Ferretti et al. (Chapter 1). In short, we amplified 12 highly polymorphic microsatellite regions (Table 2.1) through polymerase chain reactions in a DYAD thermal cycler (Bio-Rad) from DNA extracted from the blood samples collected in the field, following the conditions in Makarewich et al. (2009). PCR products were then genotyped on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), and the sizes of the microsatellite alleles estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems). We genotyped a total of 342 nestlings and their social parents (Ferretti et al. Chapter 1), and assigned paternity using the maximum likelihood approach in the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Allele frequencies and observed (H_O) and expected heterozygosity (H_E) at each locus were calculated with the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007).

Table 2.1 Microsatellite loci used in this study (Makarewich et al. 2009). N: number of unrelated individuals genotyped, N_A: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity.

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

Measures of reproductive success

We used clutch size and number of young fledged as measures of reproductive performance. We did not consider nests that failed during incubation in our comparisons of number of young fledged between groups. Only nests that hatched young could be sampled for paternity analyses, and hence, for these nests we have measures of both clutch size and number of young fledged. To compare observed

differences in clutch size and number of young fledged between nests with different paternity status we used one-way ANOVAs. We run two ANOVA's for each variable. In one analysis nests were separated into two groups: nests with extra-pair young and nests with no extra-pair young. Because of the high incidence of nests with all extra-pair young in our population (see Ferretti et al. Chapter 1) we subdivided nests with extra-pair young into two categories, resulting in three groups for this second comparison: nests with all extra-pair young in them (ALL EPY), nests with all within-pair young in them (ALL WPY), and broods of mixed paternity (MIXED).

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

Measures of heterozygosity and genetic compatibility

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

We calculated relatedness between the breeding adults using the program KINGROUP v2 (Konovalov et al. 2004) and used this value as a measure of genetic similarity of the pair. The average genetic similarity of our sampled adult population was -0.0089 (expected value is 0, Stapleton et al. 2007). We used binary logistic regressions to test the ability of female H_{ST}, social male H_{ST} and genetic similarity between the social breeding pair to explain EPP status (presence or absence of extra-pair young at any one nest).

We examined the correlation between offspring heterozygosity and genetic similarity of the genetic parents by using Pearson's coefficient (for EPY we used only those cases for which we were able to assign a genetic father to the offspring). For those cases in which we could identify the extra-pair sire, we compared the genetic similarity of the social dyad (i.e. genetic similarity between the female and her social mate) with that of the extra-pair dyad (i.e. female with the extra-pair male) with a paired *t*-test. We used a mixed linear model (REML estimation), with nest as a random factor to control for maternal effects, to compare H_{ST} of extra-pair and within-pair young, as well as H_{ST} of offspring that died and those that survived. We also compared H_{ST} of nestlings from different nest types (i.e nests with all EPY, nests with all WPY and mixed paternity broods) using a mixed linear model (REML estimation) with nest as a random factor.

Statistical analyses

Analyses were run on JMP 8.0.1 (SAS Institute Inc. 2009) and SPSS 14 (SPSS Inc. 2005).

Results

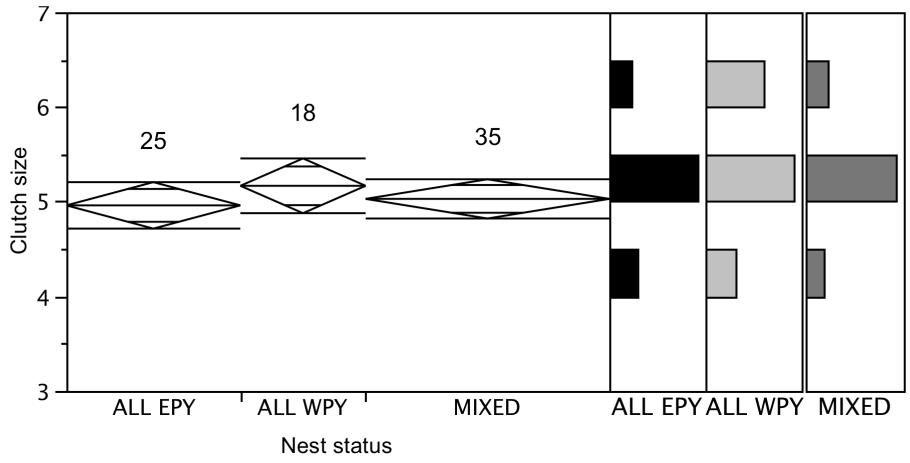
Reproductive success and extra-pair paternity

We found that 77% of broods had extra-pair young (60/78 nests) and 56% of the nestlings (193/342) in the population were a result of extra-pair matings (Ferretti et al. Chapter 1). We were able to identify the extra-pair sires of 90 of these 193 offspring, corresponding to 31 males.

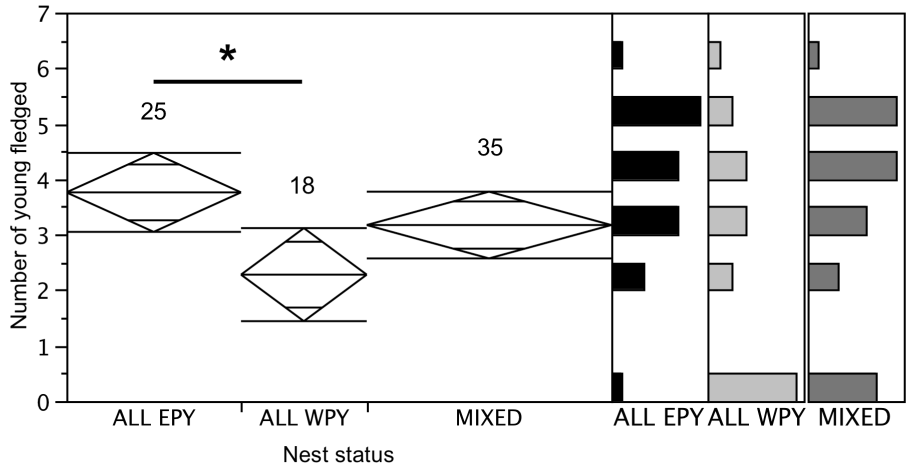
Differences in clutch size for nests with and without extra-pair young were not significant ($F_{1, 76} = 2.008$, $p = 0.160$, power = 0.903), but nests with at least one EPP offspring fledged overall more young than did those without EPP offspring ($F_{1, 76} = 7.982$, $p = 0.006$). This pattern did not change when we divided the nests in three categories (ALL EPY, ALL WPY and MIXED, Figure 2.1A). Clutch size did not differ across groups ($F_{2, 75} = 0.597$, $p = 0.552$, power = 0.126), but the number of young fledged differed across groups ($F_{2, 75} = 3.608$, $p = 0.032$, Figure 2.1B). We conducted *post hoc* comparisons to identify differences between categories and found that ALL EPY nests significantly fledged more young than ALL WPY nests (Student's *t*-test $p = 0.008$), but we did not find differences between the other pairs (MIXED-ALL WPY Student's *t*-test $p = 0.088$; ALL EPY-MIXED Student's *t*-test $p = 0.212$). EPY had a greater probability of surviving than did WPY when all nests were considered (Fisher's exact test $p < 0.001$, $N = 342$; Figure 2.2), but when only nests with mixed broods were used in the analyses we failed to find a significant difference in probability of survival with respect to extra-pair paternity status (Fisher's exact test $p = 0.591$, $N = 153$), suggesting that the difference detected when all nests were considered was more likely an effect of a nest-wide effect rather than the extra-pair status of individual offspring *per se*.

Figure 2.1 Reproductive performance differences between nests with extra-pair offspring (ALL EPY and MIXED) and nests with only within-pair offspring (ALL WPY). The y-axis represents clutch size (A), and number of young fledged (B). The diamond width represents the range of the data. The horizontal line in the middle of the diamond is the group mean; the first lines above and below the mean represent the standard errors, and the next lines are the 95% confidence intervals. Sample sizes for each group are shown above the diamonds. Asterisk represents a significant difference ($\alpha = 0.05$). The panel on the right shows the histograms for the number of cases in each category. Bars are proportional within, but not, across groups. Results presented as mean ± standard errors. A) Mean clutch size ALL EPY: 4.96 ± 0.12, ALL WPY: 5.16 ± 0.14, MIXED: 5.03 ± 0.10; B) Mean number of nestlings fledged ALL EPY: 3.76 ± 0.35, ALL WPY: 2.28 ± 0.42, MIXED: 3.17 ± 0.30.

A)


Clutch size
7
6
5
4
3
25
18
35
ALL EPY
ALL WPY
MIXED
ALL EPY
ALL WPY
MIXED
Nest status

B)


Number of young fledged
7
6
5
4
3
2
1
0
*
25
18
35
ALL EPY
ALL WPY
MIXED
ALL EPY
ALL WPY
MIXED
Nest status

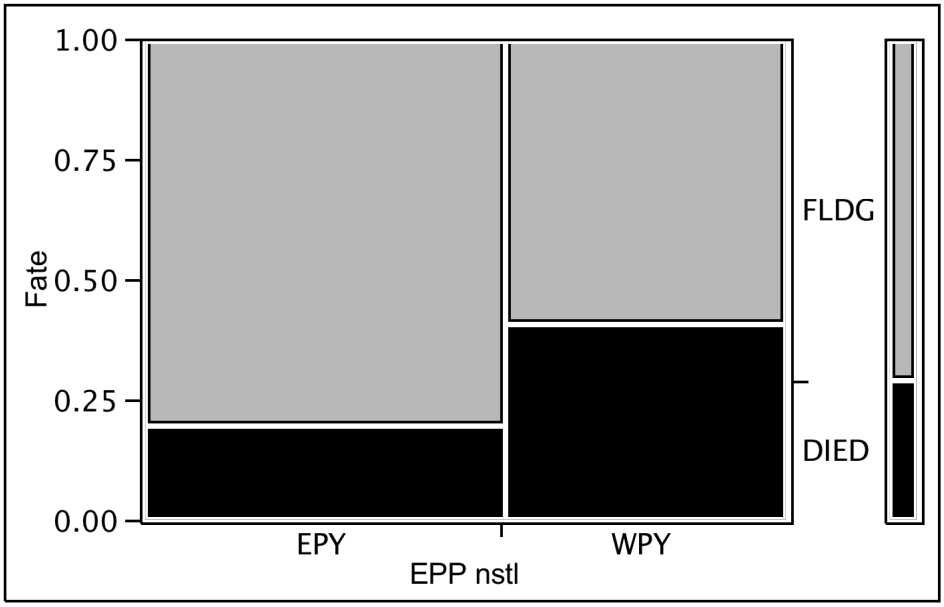


Figure 2.2 Mosaic plot for the proportion of nestlings that survived and died divided by the nestlings' paternity status. The y-axis represents the probability of a nestling surviving or dying. The column on the right represents the expected proportions for each category calculated by the contingency test. EPY: extra-pair young, WPY: within-pair young, FLDG: nestlings that fledged successfully, DIED: nestlings that died.

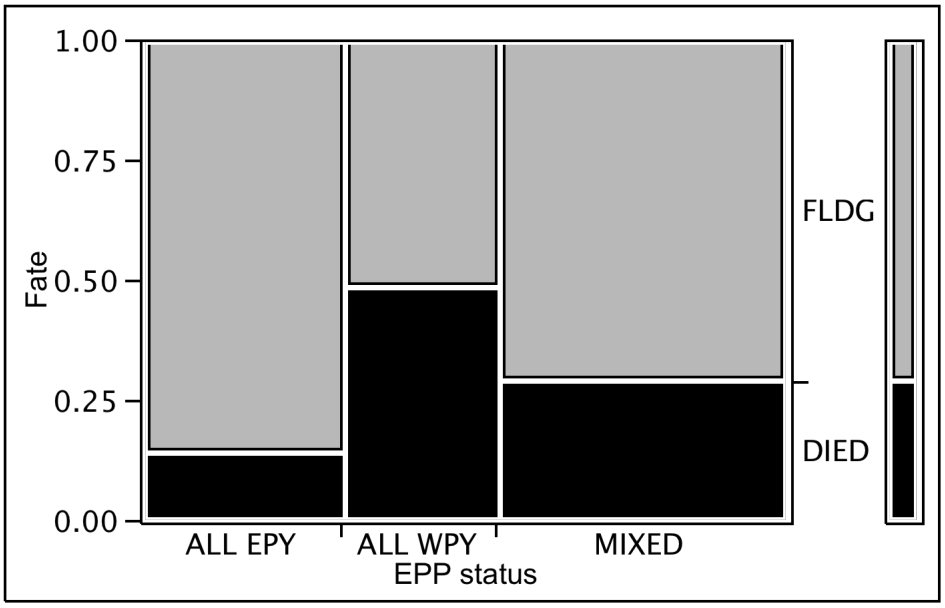


Figure 2.3 Mosaic plot for the proportion of nestlings that survived and died divided by nest paternity status. The y-axis represents the probability of a nestling surviving or dying. The column on the right represents the expected proportions for each category calculated by the contingency test. ALL EPY: broods containing all extra-pair offspring, ALL WPY: broods containing all within-pair offspring, MIXED: broods containing extra-pair and within-pair young, FLDG: nestlings that fledged successfully, DIED: nestlings that died.

The probability of a nestling fledging differed among groups and depended on the status of the nest where the nestling was raised. Nests with all within-pair young had the lowest probability of surviving, and young in nests with all extra-pair offspring had the highest per-chick probability of surviving ($\chi^2 = 27.591$, $p < 0.001$, N = 342, Figure 2.3).

Heterozygosity and genetic similarity

For the analysis of heterozygosity we only used pairs once (i.e. we did not use the information on the second broods of pairs that re-nested) and we only used individuals that were typed at 8 or more loci. This resulted in a total of 65 nests. We did not find female H_{ST} or social male H_{ST} to be good predictors of EPP status (Female: $\chi^2 = 0.78$, $p = 0.376$, power =0.938; Male: $\chi^2 = 1.79$, $p = 0.180$, power =0.910), nor did genetic similarity between the members of the social pair successfully predict EPP status ($\chi^2 = 0.27$, $p = 0.605$, N = 65 dyads, power =0.496). As expected, the H_{ST} of an offspring was negatively related to the genetic similarity of its genetic parents (Pearson's $r = -0.247$, $p < 0.001$, N = 223).

We were able to compare the genetic similarity of the social pair to the genetic similarity of the female and extra-pair male for those cases in which we could identify extra-pair sires. Extra-pair males sometimes sired more than one nestling in a single brood, and some sired nestlings in different broods. Thus, for those that sired multiple nestlings with one female, we only used one case for that nest, but for those that sired nestlings in different broods we took a measure of genetic similarity of that male with each of the females with whom he sired offspring. Whenever possible, we formed "dyads" of all female's known matings (female with extra-pair male vs. female with social mate) for comparison. Although mean genetic similarity between the social pair was smaller (i.e. the female and the social male were more genetically different) than the genetic similarity of the female and the extra-pair partners (paired *t*-test, $t = 3.868$, $p < 0.001$, N = 34), there was no significant correlation between the measures of genetic similarity within the dyads (Pearson's $r = -0.075$, $p = 0.675$, power = 0.364, N = 34).

Extra-pair offspring and within-pair offspring did not differ in their level of H_{ST} ($F_{1, 247} = 1.175$, $p = 0.279$, power = 0.586, Figure 2.4), but offspring that did not survive had a higher H_{ST} than did the ones that fledged ($F_{1, 147} = 5.181$, $p = 0.024$, Figure 2.5). We found similar patterns when only nests of mixed paternity were considered for the analysis ($F_{1, 135} = 0.0004$, $p = 0.983$; $F_{1, 64} = 4.020$, $p = 0.049$, respectively). We did not find significant differences in the level of H_{ST} of the nestlings in broods of different paternity status ($F_{2, 73} = 0.904$, $p = 0.410$, power = 0.327, Figure 2.6).

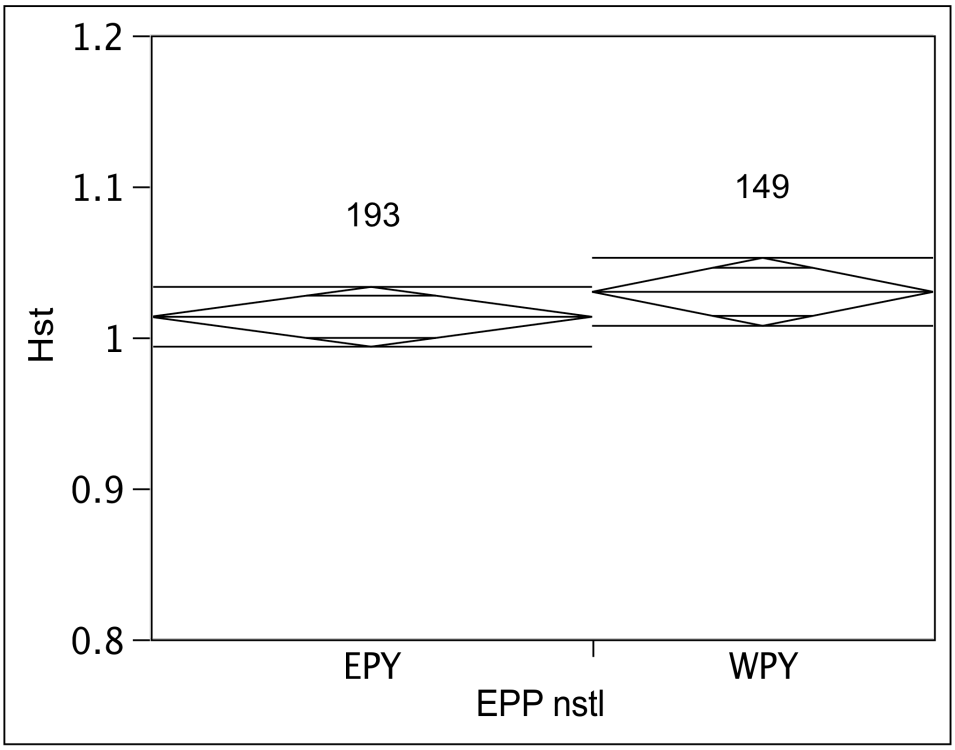


Figure 2.4 Standardized heterozygosity for extra-pair and within-pair nestlings. The y-axis represents the standardized heterozygosity, and the x-axis represents the offspring paternity status. The diamond width represents the range of the data. The horizontal line in the middle of the diamond is the group mean; the first lines above and below the means represent the standard errors, and the next lines are the 95% confidence intervals. Sample sizes for each group are shown above the diamonds. Hst: Standardized heterozygosity, EPY: extra-pair young, WPY: within-pair young. Results presented as mean ± standard errors. Mean standardized heterozygosity EPY: 1.01 ± 0.01, WPY: 1.03 ± 0.01.

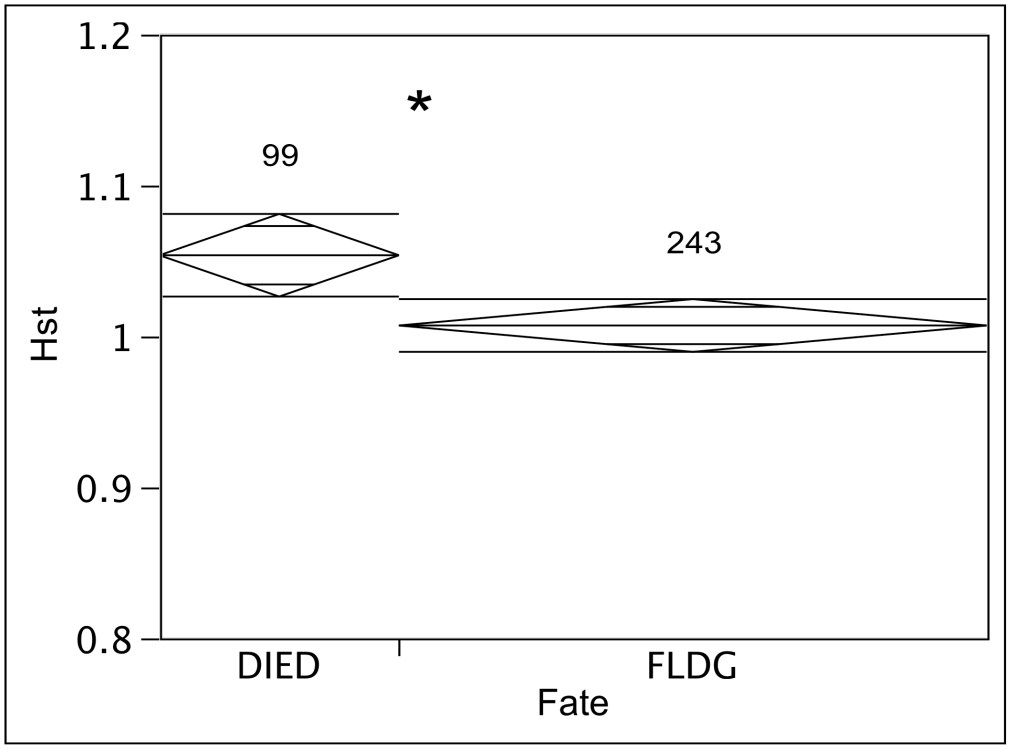


Figure 2.5 Standardized heterozygosity for nestlings that died and those that fledged. The y-axis represents the standardized heterozygosity, and the x-axis represents the offspring's fate. The diamond width represents the range of the data. The horizontal lines in the middle of the diamonds are the group means; the first lines above and below the means represent the standard errors, and the next lines are the 95% confidence intervals. Sample sizes for each group are shown above the diamonds. Hst: Standardized heterozygosity, DIED: nestlings that died in the nest, FLDG: nestlings that survived. Asterisk represents a significant difference ($\alpha = 0.05$). Results presented as mean ± standard errors. Mean standardized heterozygosity DIED: 1.05 ± 0.01, FLDG: 1.01 ± 0.01.

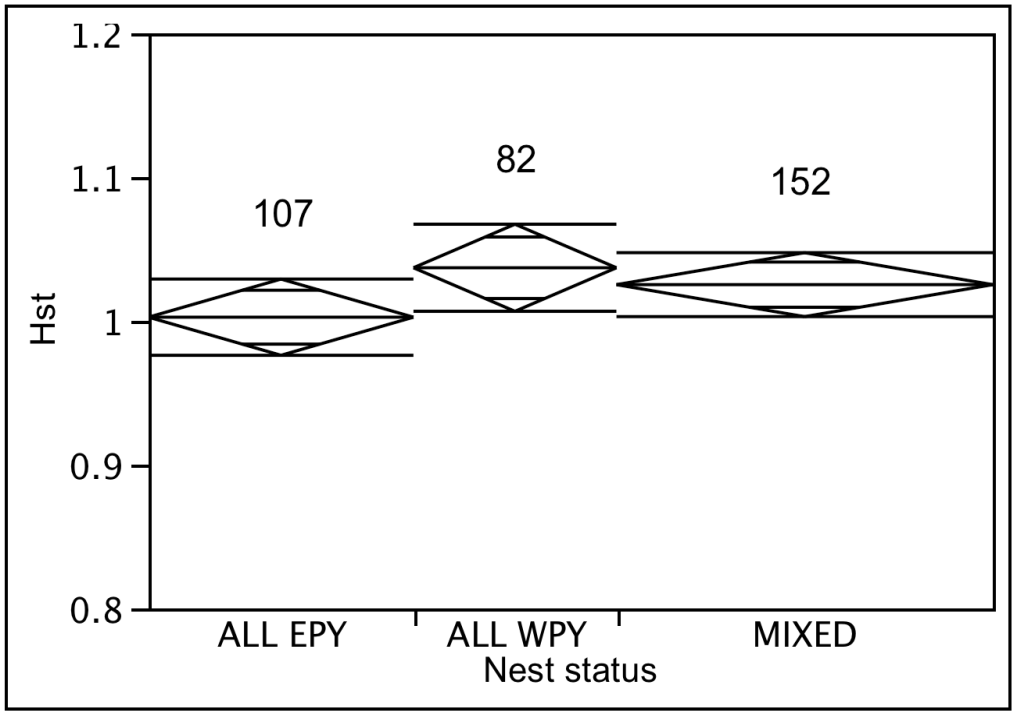


Figure 2.6 Standardized heterozygosity for nestlings in different nests. The y-axis represents the standardized heterozygosity, and the x-axis represents the nest status in relation to the paternity of the brood. The diamond width represents the range of the data. The horizontal lines in the middle of the diamonds are the group means; the first lines above and below the means represent the standard errors, and the next lines are the 95% confidence intervals. Sample sizes for each group are shown above the diamonds. Hst: Standardized heterozygosity, ALL EPY: broods with all extra-pair offspring, ALL WPY: broods with all within-pair offspring, MIXED: broods of mixed paternity. Results presented as mean ± standard errors. Mean standardized heterozygosity ALL EPY: 1.00 ± 0.01, ALL WPY: 1.04 ± 0.01, MIXED: 1.03 ± 0.01.

Discussion

Extra-pair paternity and reproductive success

Although female White-rumped Swallows that have EPY in their nests did not lay significantly different numbers of eggs than females that have all WPY, the former fledged more young (Figure 2.1). There are three possible interpretations for this result. First, females that have extra-pair young might be better at providing parental care, and thus their nestlings do better and survive more. This would be consistent with the "constrained female hypothesis" proposed by Mulder et al. (1994) and Gowaty (1996) that suggests that females that are better parental care providers, and can potentially suffer the costs of reduced male care, should be the ones to engage in extra-pair behavior. A second alternative to these results could be that males mated to the females that engage in extra-pair behavior are good parental care providers—which might mean that they spend more time defending the nests from intruders and predators and/or they are better than other males at providing food for the nestlings. If males spend more time at their nests, their females might be able to judge through behavioral cues the quality of their social mate as a parent, and even engage in extra-pair copulations during their fertile period more freely, given the open opportunity for increased chances for undisturbed copulations. A last alternative may indicate that these differences are driven by some fitness advantage of the extra-pair offspring. We can divide these alternatives into two more inclusive categories: males and/or females are driving these differences in survival by being better parents, or these differences reflect the quality of the extra-pair offspring.

In order to differentiate between these two major alternatives we compared the fate of EPY and WPY. EPY had a higher probability of surviving the period at the nest than WPY (Figure 2.2). Similarly, nests with all EPY had the highest probability of

fledging young and nests with all WPY, the lowest (Figure 2.3). However, when only nests of mixed paternity were used in this comparison we failed to find differences in survival between EPY and WPY. This suggests that, in nests where there are one or several EPY, the social parents might be better parental care providers than the social pair in nests with all WPY.

Heterozygosity, paternity and reproductive success

Under the heterozygosity theory (Brown 1997) a female's mating strategy should be to mate with males having alleles that best complement her own and thus increase offspring heterozygosity—and, as a result, offspring fitness. Under this scenario the female's own heterozygosity should predict mating behavior. That is, females with low heterozygosity levels would preferentially mate with one or more males that complement her genetic composition. Similarly, the social male's heterozygosity should also be a predictor of the mating strategy of his partner. Males that are more homozygous should have extra-pair offspring in their nests. In addition, the genetic similarity between the social pair should predict mating behavior: females should engage in extra-pair behavior more often in those cases in which the pair is genetically similar. In our analyses we found that neither male nor female heterozygosity nor the genetic similarity between the nest-attending adults were good predictors of EPP status. However, when we compared the difference in genetic similarity between the female and her social mate to that of the female with her extra-pair mate we did find significant differences—females chose extra-pair partners with whom they shared a higher degree of genetic similarity, when compared to their social partners.

One of the predictions made by the heterozygosity theory (Brown 1997) is that more heterozygous offspring should have a higher fitness, and that EPY should be

more heterozygous than WPY, after controlling for the shared maternity at the nest. However, we found that EPY and WPY did not differ in their level of heterozygosity, and nestlings that died before fledging were more heterozygous than those that fledged. Given the negative correlation between the offspring's heterozygosity level and the genetic similarity of the parents, how is it possible, then, that EPY and WPY do not differ in heterozygosity, but extra-pair males and within-pair males do differ in their degree of genetic similarity with the social female? One explanation may be that females engage in many extra-pair copulations with the available males, but that fertilization success might bias the outcome of such copulations (Griffith and Immler 2009). Zeh and Zeh (1997, 2008) suggest that the female's reproductive tract provides a physiologically hostile environment where incompatible sperm and embryos are screened (e.g. failed fertilization, early interruptions in the development of the embryo, etc.). In such cases, selection on heterozygosity will occur at the gamete or embryo level, and could result in EPY and WPY having similar levels of heterozygosity (Zeh and Zeh 2008), even if females prefer to mate with extra-pair males that are more similar to themselves (as shown here) or more different (as in other studies) in response to selective pressures in the environment.

The heterozygosity hypothesis for female mate choice remains a controversial topic (reviewed in Akçay and Roughgarden 2007 and Wetzel and Westneat 2009), but there seems to be growing evidence that selection might drive females to avoid the costs of inbreeding by selecting extra-pair males that increase the heterozygosity of their offspring (Zeh and Zeh 2008, Griffith and Immler 2009). Our results, however, contradict the many predictions made by this hypothesis (Brown 1997, see predictions in Table 1 in Wetzel and Westneat 2009): social males are less related to the females

than extra-pair males and offspring that died were more heterozygous than those that survived.

In many ways, the patterns in *T. leucorrhoa* appear to be the mirror image of those from a similar study conducted on its congener, *T. bicolor*, in Canada (Stapleton et al. 2007). In that study the authors found that EPY were more heterozygous than WPY, but social mates and extra-pair mates did not differ in their levels of genetic similarity with the female, and the genetic similarity with the social mate did not predict the presence of EPY. In addition, they did not find a difference in heterozygosity between offspring that died and those that fledged. The authors also found that the more heterozygous EPY were a result of copulations outside of the breeding colony. Thus, the situation in *T. bicolor* appears to be opposite that in *T. leucorrhoa:* females increased offspring heterozygosity through extra-pair matings, despite the lack of difference in the female's genetic similarity between extra-pair and within-pair mates, and there did not seem to be an obvious fitness effect on more heterozygous offspring.

Conclusions

White-rumped swallows readily engage in extra-pair matings that result in a high proportion of EPY, about half of which are sired by males within the colony limits (Ferretti et al. Chapter 1). Despite the seeming failure of heterozygozity to explain extra-pair mating in this species, there remain interesting patterns to be interpreted. We found a fitness advantage for nests that had EPY—as these nests fledged more young—but this advantage seems more likely due to the adults at those nests being better at providing parental care or interacting in some way differently from those that had only WPY.

Consequently, it appears that *T. leucorrhoa* exhibits a system where heterozygosity increase is not the selective outcome of extra-pair female mate choice, and they present a pattern that is thus the antithesis of what has been found in *T. bicolor*. Further study of the reproductive behavior of both sexes in this species might provide an interesting insight into this subject. Are mixed- and extra-pair brood females better females that are able to compensate for any potential retaliation from their cuckolded mates (Gowaty 1996), or are cuckolded males for some reason more willing or able to contribute parental care? These and many other questions remain before us, and they remind us that different species under different ecological conditions and with different evolutionary heritages might show completely different modes of mate choice and parental interactions. We can expect to uncover more diverse patterns and hope for new generalities in the more detailed data sets that more carefully phylogenetically controlled fieldwork will produce in the future.

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CHAPTER 3

FROM TIERRA DEL FUEGO TO BRITISH COLUMBIA: VARIATION IN EXTRA-PAIR PATERNITY RATES ACROSS FIVE SPECIES OF *TACHYCINETA* SWALLOWS

Abstract

Extra-pair paternity rates vary markedly across avian taxa, but patterns of variation in this trait have been confounded by a paucity of data on closely related species, especially those spanning broad environmental gradients. Here we compare variation in extra-pair paternity rates among five species in the widespread swallow genus *Tachycineta*. Rates of extra-pair paternity vary widely in this group, ranging from 12 to 89% of nests having extra-pair young. The interspecific variation in extra-pair paternity within this small group of closely related swallows has a range equivalent to that found among all Hirundinidae, and is close to the range of variation across all birds. Despite theory that predicts similar extra-pair paternity rates in species that occupy similar environments or which are closely allied phylogenetically, one of the most striking contrasts in rates of extra-pair paternity within *Tachycineta* occurs between the two southern hemisphere sister species. More generally, extra-pair paternity rates in these swallows do not closely track a latitudinal gradient, as predicted by studies of other life-history traits. Extra-pair paternity is connected to other life-history traits through a complex network of trade-offs, which in turn can be affected by ecological and environmental factors, and this might be the main reason why understanding variation in genetic mating systems remains a difficult task.

Introduction

Broad geographic patterns have long intrigued researchers interested in the evolutionary and ecological determinants of variation in life-history traits (see Ricklefs 2000 for a review). Early contributors to the development of avian life-history theory documented latitudinal clines in life-history characters and provided a variety of ecological hypotheses to explain this variation (Moreau 1944, Lack 1948, Skutch 1949), suggesting that variation in life-history strategies is related to current ecological factors that co-vary with environmental heterogeneity. More recently, careful comparative analyses (Owens and Bennett 1995, Bennett and Owens 2002) conducted on 250 species of birds concluded that most of the variation in life-history traits in Aves arose early in the diversification of birds at the level now associated with families and orders. These indications of an early evolutionary radiation of life-history strategies suggest in turn that the variation observed today has responded most strongly to ancient ecological selective factors that played a key role in the radiation of birds.

Extra-pair paternity (EPP) is considered a life-history trait, as it influences both male and female reproductive success (Mauck et al. 1999, Bennett and Owens 2002). Although most bird species are socially monogamous (Lack 1968), molecular techniques have revealed large variation among avian taxa in the proportion of broods that have extra-pair young (0-95%, Arnold and Owens 2002). A number of hypotheses have been proposed to explain inter-specific variation in EPP rates in birds as a result of extra-pair copulations (EPCs; reviewed in Bennett and Owens 2002, Griffith et al. 2002, Neudorf 2004). However, a comparative study on variation in EPP rates on 88 species of birds showed that this trait follows the same pattern of ancient diversification found in other life-history traits (Arnold and Owens 2002, Owens and

Bennett 1995), with more than 50% of the inter-specific variation being explained by differences at the taxonomic level of families and orders. Yet, substantial variation exists in EPP rates below the level of families (Westneat and Stewart 2003) suggesting that there is still not a clear understanding of the current factors influencing mating systems and the interaction between these and the evolutionary history of the taxa under consideration. One limitation of comparative studies of variation in EPP is that there are very few closely related groups of species that can be included in the analysis. Data are lacking on the genetic mating system for multiple species within nearly all genera or most families, especially those spanning pronounced environmental gradients, making generalizations difficult. For example, in Arnold and Owens' (2002) rigorous comparison of 88 species of birds distributed among 36 families, only two genera (*Falco* and *Parus*) had more than two species represented. There is likewise a marked geographic bias, as most of these species have been sampled from populations in Europe and/or North America (the holarctic region).

We explore here the genetic mating system of five species in the swallow genus *Tachycineta* (*T. bicolor*, *T. thalassina*, *T. albilinea*, *T. leucorrhoa* and *T. meyeni*) that span a wide breeding distribution in North, Central and South America. Previous studies based on two species in this genus have suggested that *Tachycineta* swallows show high variation in their degree of extra-pair paternity (Moore et al. 1999), with 26% of nests with extra-pair young in *T. albilinea* (Moore et al. 1999) and up to 89% of nests having at least one extra-pair young in *T. bicolor*—one of the highest rates found in any socially monogamous passerine (Dunn et al. 1994, Barber et al. 1996, Kempenaers et al. 1999, Whittingham & Dunn 2001, O'Brien & Dawson 2007, Stapleton et al. 2007, Crowe et al. 2009). The overall ecological similarities of these species, the marked environmental gradient they inhabit, and their large

differences in genetic mating system make this genus a compelling group for studying variation in EPP rates in birds.

In addition, an advantage of using *Tachycineta* swallows in cross-species analyses is that the evolutionary relationships of all species within this genus and its relatives have been well characterized through a series of molecular phylogenetic studies. These analyses have identified a single congruent and well-supported tree based on independent molecular markers (mtDNA: Sheldon et al. 1999, Whittingham et al. 2002, Sheldon et al. 2005; nuclear introns: Sheldon et al. 2005). The genus *Tachycineta* consists of two major clades: one comprising North American and Caribbean species (*T. bicolor*, ((*T. thalassina*, *T. euchrysea*), *T. cyaneoviridis*)), and one comprising South and Central American species ((*T. stolzmanni*, (*T. albilinea*, *T. albiventer*)), (*T. leucorrhoa*, *T. meyeni*)) (Whittingham et al. 2002). These reconstructions also confirm that *Tachycineta* is a monophyletic group within the larger swallow radiation, and provide a solid framework for historical analyses of life-history traits.

In this study, we more fully document interspecific variation in EPP among *Tachycineta* swallows by i) characterizing for the first time the genetic mating system for two swallow species: *T. meyeni* and *T. thalassina*; ii) describing the rate of EPP in two additional populations of *T. albilinea* and *T. leucorrhoa*; iii) mapping variation in the extra-pair mating system on the phylogenetic tree of the genus; and iv) examining the geographic pattern of variation in EPP rates. This analysis of variation among closely related *Tachycineta* species allows us to simultaneously evaluate diversification in EPP rates from a historical and a contemporary ecological standpoint. If phylogenetic history is the main driver of genetic mating system

variation, closely related species (e.g., sister taxa) should have similar levels of EPP. However, if current environmental/ecological variables are dynamic selective pressures shaping current mating system differences, then species experiencing similar conditions (e.g., breeding at similar latitudes) should have similar EPP rates regardless of their evolutionary history.

Materials and methods

Colonies and species

The nine species in the New World genus *Tachycineta* are distributed throughout the Americas and the Caribbean (Turner and Rose 1989). In this study we included five of the species with continental distributions, with samples spanning a range of latitudes from 53°N (British Columbia) to 54°S (Tierra del Fuego). We used previously reported paternity and latitudinal data for three populations of *T. bicolor* in the North, one population of *T. albilinea* in the tropics and one population of *T. leucorrhoa* in the South (see Table 3.2 for citations). We generated new information for two previously uncharacterized species—a population of *T. meyeni* from Tierra del Fuego, and two populations of *T. thalassina* from the Western United States—and for an additional population of *T. albilinea* in Central America and an additional population of *T. leucorrhoa* in Buenos Aires. Table 3.2 provides details on the locations of the breeding colonies used in this analysis. Swallows were studied from 2007-2009 for *T. meyeni*; 2003-2004 for *T. leucorrhoa*; 2003 for *T. albilinea*; and 2008-2009 for *T. thalassina* breeding near 38ºN and 2004 for the breeding colony located near 44ºN.

Standardized field protocols for sampling

Details on sampling protocols and nest-box spacing for the previously reported populations of *T. bicolor*, *T. albilinea* and *T. leucorrhoa* can be found in the references

in Table 3.2. For all other colonies used in our study, nest-boxes are spaced at 20-35m distances and checked every other day for the length of the breeding season. We captured both adult breeders inside the nest boxes using box traps for every nesting attempt (see http://golondrinas.cornell.edu for details on boxes and traps). All captured individuals were measured, bled, and banded with aluminum bands. When nestlings were 7-9 days old, we banded them with aluminum bands and took a blood sample from each. We took 20-70µl of blood from both adults and nestlings, collected using a heparinized capillary tube via brachial venipuncture, and then stored whole blood in Queen's lysis buffer (Seutin et al. 1991). When nestlings were found dead in the nest before they were banded and bled, we collected a sample from their pectoral muscle and stored it in 96% ethanol.

Genetic analyses for each of the species considered

We extracted DNA from blood and muscle samples using DNA purification kits by Eppendorf and Qiagen. Extracted DNA was diluted 1:10 in ultrapurified H_2O and then amplified at a panel of highly polymorphic microsatellite loci (Crossman 1996, Dawson et al. 2000, Stenzler 2001, Makarewich et al. 2009), which differed somewhat across species (see Table 3.1 for combination of loci used for each species and primer concentrations; conditions for *T. leucorrhoa* followed Ferretti et al. Chapter 1). We amplified multiple loci in multiplexed polymerase chain reactions (PCR); this allowed us to score all loci with only three to four PCR reactions per individual. The combination of primers used in each of these multiplexed reactions was selected by using primers with unique fluorescent dyes and to avoid fragment-size overlap in the PCR products. PCRs were performed in 10µl final volumes. Each of the mixes used 10-100ng DNA, 10mM Tris-HCl (ph 8.3), 50mM KCl, 3.25mM $MgCl_2$, 200µM

dNTPs (Invitrogen), 0.25U Jumpstart Taq polymerase (Sigma), the specified mix of forward and reverse primers, and H_2O to bring the final volume to 10µl.

PCRs were performed in a DYAD thermal cycler (Bio-Rad). Cycling profiles followed one incubation cycle of 95ºC for 2min; 35 cycles of 50s at 95ºC, 1min at the annealing temperature (see Table 3.1), and an extension time of 1min at 72ºC; these 35 cycles were followed by a final extension phase of 30min at 72ºC. PCR products were then genotyped on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), and the sizes of the microsatellite alleles estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems).

Allele frequencies and population genetic parameters were generated using the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). We assessed paternity for the populations studied using the microsatellite profiles generated by the program GeneMapper using Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007), a likelihood-based method. This program calculates the probability of paternal exclusion when one parent is known (in our case the mother) for each locus. The combined exclusion probability for all loci combined for each species was > 0.9999.

Table 3.1 Microsatellite primers used for genotyping *T. albilinea*, *T. meyeni* and *T. thalassina*. N: number of unrelated individuals genotyped, Na: number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, Ann Temp: annealing temperature. References: (a) Makarewich et al. 2009; (b) Richardson et al. 2000; (c) Crossman 1996; (d) Dawson et al. 2000; (e) Stenzler 2001

Species	**Locus**	**N**	**Na**	**Ho**	**He**	**Ann Temp**	**Primer concentration**	**Reference**
T. meyeni	Tabi1	56	8	0.857	0.783	56	1.2pM	(a)
	Tabi4	56	8	0.768	0.731	56	4.8pM	(a)
	Tal6	56	4	0.268	0.254	58	1.6pM	(a)
	Tal8	56	35	1.000	0.964	58	3.6pM	(a)
	Tle17	56	6	0.500	0.502	56	2.4pM	(a)
	Tle19	56	4	0.446	0.411	56	1pM	(a)
	Tle21	56	4	0.250	0.229	58	1.2pM	(a)
	Tle4	56	25	0.946	0.947	56	2.4pM	(a)
	Tle8	56	8	0.714	0.690	56	1.2pM	(a)
	Tle11	56	18	0.804	0.745	60	1.2pM	(a)
T. albilinea	Tabi1	48	8	0.563	0.561	56	1pM	(a)
	Tabi8	48	9	0.604	0.805	56	3.6pM	(a)
	Tle19	48	6	0.771	0.675	56	1pM	(a)
	Tle4	48	15	0.792	0.853	56	2.4pM	(a)
	Tle11	60	21	0.708	0.939	60	2pM	(a)
	Tal7	48	13	0.771	0.808	58	2.4pM	(a)
	Tal8	48	11	0.771	0.744	58	2pM	(a)
	Tle14	48	10	0.729	0.660	60	3pM	(a)

Table 3.1 (continued)

Species	Locus	N	Na	Ho	He	Ann Temp	Primer concentration	Reference
T. thalassina	Tabi1	20	8	0.842	0.878	56	1.2pM	(a)
	Tabi8	20	7	0.789	0.748	56	3.6pM	(a)
	Tal11	20	5	0.357	0.324	58	1.6pM	(a)
	Tal6	20	5	0.474	0.649	58	3pM	(a)
	Tal7	20	23	0.929	0.984	58	1.2pM	(a)
	Tle16	20	7	0.684	0.698	56	1.2pM	(a)
	Tle19	20	11	0.842	0.871	56	1.2pM	(a)
	Ase29	20	4	0.600	0.572	56	1.2pM	(b)
	MP3-31	20	12	0.800	0.908	56	1.2pM	(c)
	MP5-29	20	3	0.250	0.229	60	1.2pM	(c)
	Pca3	20	4	0.300	0.276	56	2.4pM	(d)
	Tbi104	20	7	0.750	0.714	60	1.4pM	(e)
	Tbi81	20	3	0.150	0.309	60	1.4pM	(e)

We first compared the nestlings' genotypes with the genotype of the adult female attending their nest (i.e., the putative mother). As expected, most nestlings shared at least one allele at each of the amplified loci with their putative mother. Two nestlings of *T. thalassina* did not match the social female at one locus; we regard these nestlings as offspring of their putative mothers, and assume this single-locus allele difference to be a result of rare mutations or genotyping errors (Fernando et al. 2001). We did not find any other mismatches in *T. meyeni* nor *T. leucorrhoa*, and no nestlings mismatched their putative mother at two or more loci. The nestlings' genotypes were

then compared to those of their putative father. We considered nestlings to be extra-pair young when they mismatched the social father's genotype at two or more loci.

Statistical analyses

We examined geographic variation in EPP in the genus *Tachycineta* by testing the correlation between absolute latitude of the colonies sampled and the rate of EPP (% of nests with extra-pair young). Tests were carried out using the program JMP 8.0.1 (SAS Institute Inc. 2009).

Results

Characterization of the genetic mating system of four Tachycineta species

Here we report for the first time rates of EPP for two *Tachycineta* swallow species, *T. thalassina* and *T. meyeni* (Table 3.2). *T. thalassina* had very high rates of EPP (67-75% extra-pair nests, 56-60% extra-pair young), similar to its northern congener *T. bicolor* (72-89% of extra-pair nests, see references in Table 3.2, Figure 3.1) and similar to those found in *T. leucorrhoa* (78% of extra-pair nests, Table 3.2), a species breeding at a similar latitude in the southern hemisphere (Figure 3.1). In contrast, we found *T. meyeni* to have the lowest rate of EPP sampled in this group, with 12% of nests having extra-pair young and only 5% of nestlings in the population being extra-pair (Table 3.2).

Historical EPP variation in the genus Tachycineta

Our results demonstrate that rates of EPP vary dramatically across *Tachycineta* species (Table 3.2, Figure 3.1), with the highest known rates in *T. bicolor* and the lowest in *T. meyeni*. Substantial variation in EPP rates in this genus can be found *between* the two major swallow clades (North American and Caribbean clade and the Central and

South American clade, Figure 3.2), as well as *within* the South and Central American clade, with the sister taxa *T. leucorrhoa* and *T. meyeni* having very different rates of EPP (Figure 3.2).

Table 3.2 Rates of extra-pair paternity for five *Tachycineta* species and latitude of populations sampled. EPP nests: % of nests with extra-pair young. EPY: % of extra-pair nestlings in the population.

Species	EPP nests	EPY	Latitude of colony	Reference
T. bicolor	82%	51%	44º34'N	(a)
	87%	49%	43°23'N	(b)
	85%	35%	53°N	(c)
T. thalassina	75% (6/8)	60% (21/35)	44º33'N	This study
	67% (18/27)	56% (44/78)	37º58'N	This study
T. albilinea	26%	15%	9º10'N	(d)
	18% (4/22)	8% (8/103)	17º36'N	This study
T. leucorrhoa	78%	55%	35º34'S	(e)
	61% (8/13)	35% (18/51)	36º25'S	This study
T. meyeni	12% (3/25)	5% (4/72)	54º44'S	This study

(a) Dunn et al. (1994), Kempenaers et al. (1999), Stapleton et al. (2007), Crowe et al. (2009). Averages for this population were calculated based on the results of these four studies.

(b) Whittingham and Dunn (2001), Whittingham et al. (2006), Dunn et al. (2009). Averages for this population were calculated based on the results of these three studies.

(c) O'Brien and Dawson (2007)

(d) Moore et al. (1999)

(e) Ferretti et al. (Chapter 1)

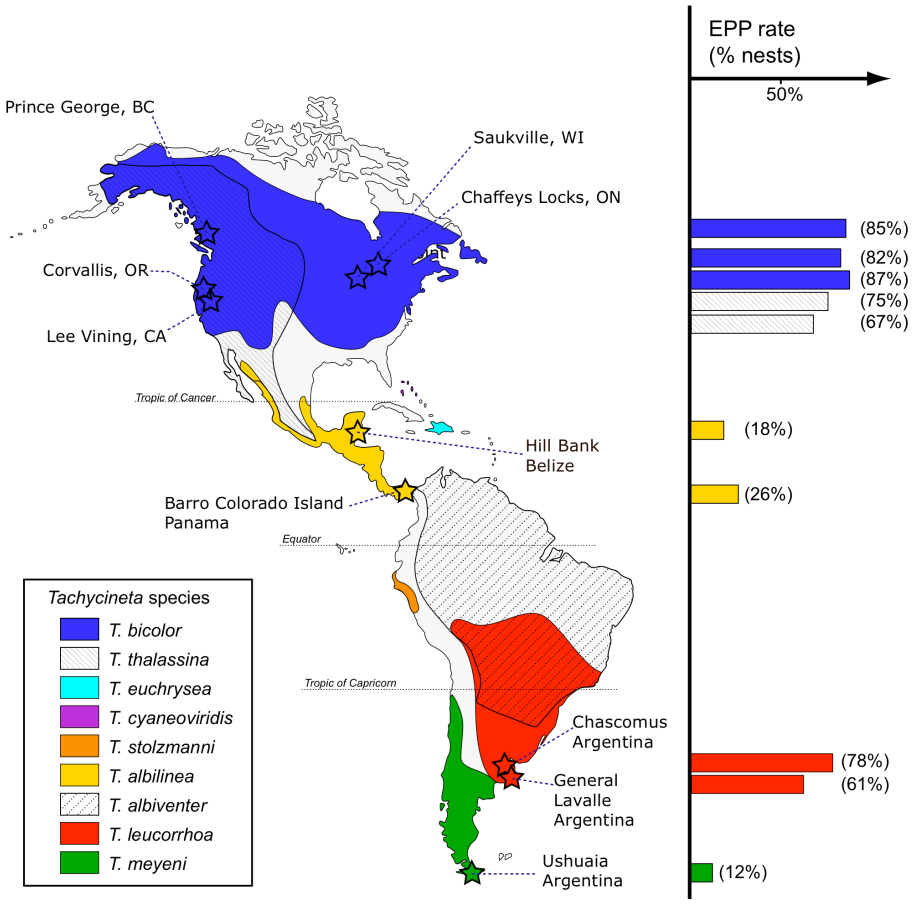

Figure 3.1 Map of the ranges of the nine species of *Tachycineta* swallows. Bars on the right represent percent of nests with extra-pair young for the colonies sampled. Bars are coded by species (see color codes) and are located at the approximate latitudes of the populations sampled.

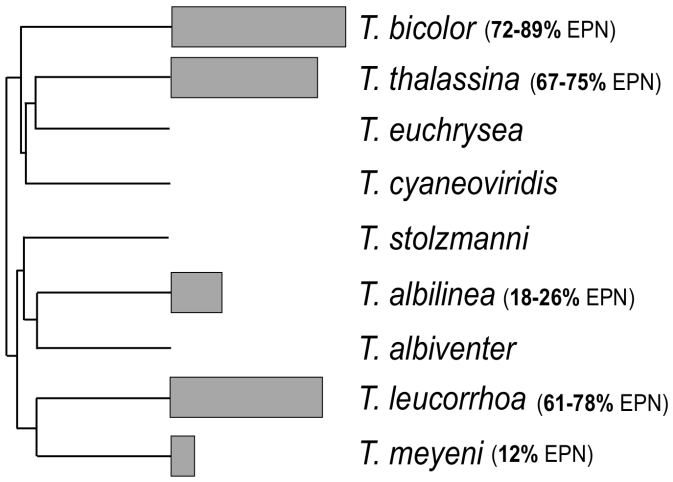

Figure 3.2 Extra-pair paternity rates across the *Tachycineta* phylogeny. Gray boxes represent the percent of nests with extra-pair young for the species surveyed. Phylogenetic relationships taken from Whittigham et al. (2002). EPN: nests with at least one extra-pair nestling.

Geographic variation in EPP rates

Genetic parentage has been proposed to vary with latitude (Stutchbury and Morton 2008), with birds in the tropics having lower rates of EPP. Yet, we did not find a significant correlation between EPP and latitude across the species and populations sampled (Pearson's coefficient = 0.463, $p = 0.178$, $N = 10$, Figures 3.1 and 3.3). However, this lack of association was highly influenced by *T. meyeni*— when we removed this species from the analysis the correlation with latitude was significant (Pearson's coefficient = 0.930, $p < 0.001$, $N = 9$).

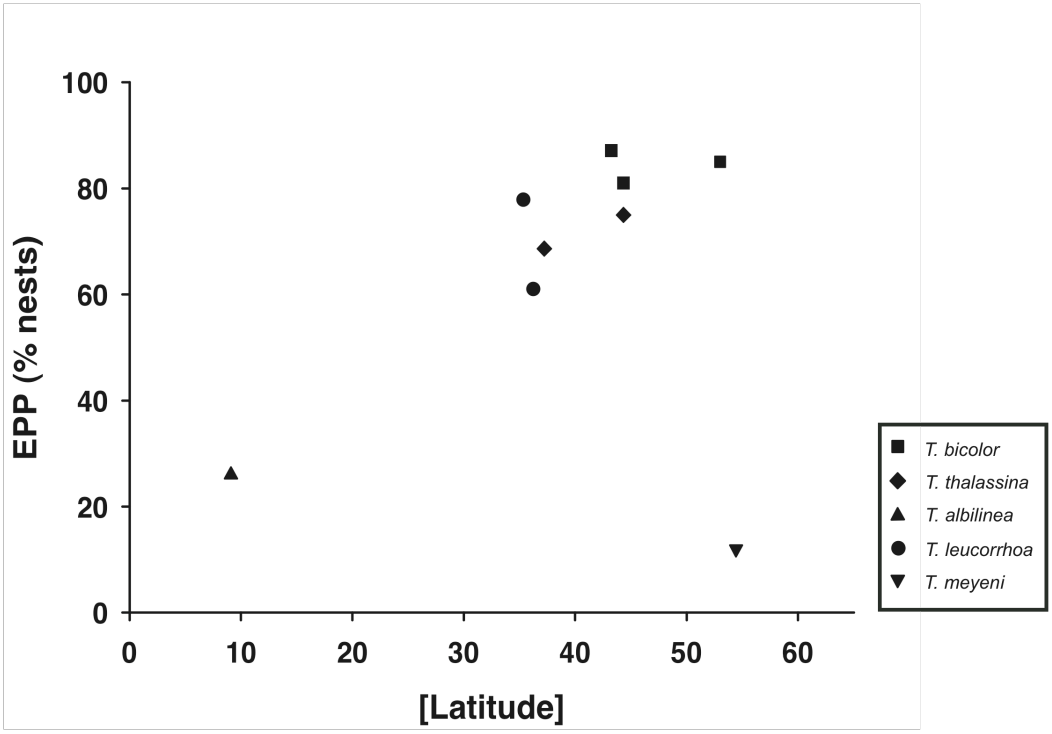

Figure 3.3 Relationship between the percent of nests with extra-pair young and absolute latitude (represented between brackets) for the eight populations of *Tachycineta* swallows sampled. Pearson's correlation coefficient = 0.463, p = 0.178.

Discussion

Understanding patterns of variation in life-history traits, and thus EPP, requires looking at within-population variation as well as variation between species and populations. Most studies on avian EPP rates to date have been restricted to single populations and single species within most genera, limiting our understanding of how evolutionary and environmental changes can affect genetic mating systems (Arnold and Owens 2002, Wilson 2009). In our study we analyzed patterns of EPP rates in five

species of *Tachycineta* swallows with populations ranging from 54°S to 53°N of latitude, from Tierra del Fuego to British Columbia. The north temperate species *T. bicolor* has long been the focus of research of behavioral ecologists, in part because of its extremely high rates of EPP (Table 3.2, Jones 2003). The contrasting finding of low rates of EPP in the tropical *T. albilinea* (Table 3.2) makes a compelling case for studying variation in EPP rates between closely related species in this genus, providing us with a unique dataset for studies of variation in paternity.

Historical EPP variation in the genus Tachycineta

We found extreme variation in the rates of EPP (12-89% broods with extra-pair young, Table 3.2, Figure 3.1), with *T. bicolor*, *T. thalassina* and *T. leucorrhoa* having the highest numbers of extra-pair broods (up to 67-89% of extra-pair nests; this study and references in Table 3.1), down to a low of 12% of extra-pair nests in the southernmost species *T. meyeni*. The extensive EPP variation found within this genus is comparable to the variation found in the entire Family Hirundinidae (see Westneat and Stewart 2003), and close to the variation found across all of Aves (0-95% Arnold and Owens 2002). It is clear from previous comparative studies (Arnold and Owens 2002, Bennett and Owens 2002) that variation in avian genetic mating patterns can have a phylogenetic component. However, the substantial variation in this trait found near the tips of the phylogenetic tree among closely related birds (Figure 3.2, see also Westneat and Stewart 2003 for a review) reminds us that we do not have yet a full understanding of when or how differentiation in EPP rates has occurred. A notable example of this variation is found between the sister taxa *T. leucorrhoa* and *T. meyeni*, with 78% and 12% of nests with extra-pair young respectively (Figure 3.2). An integrated view of the partitioning of the variance in avian mating systems across levels of relationship must await a more thorough sampling at shallow phylogenetic

levels, especially among closely related species. For example, recent work by Kingma et al. (2009) found very low levels of EPP in the Purple-crowned Fairy-wren (5.8% of the broods containing extra-pair young), a member of the genus *Malurus*, otherwise known for its high levels of promiscuity. However, this extreme change in genetic mating system within the genus *Malurus* was not associated with changes in other life-history traits hypothesized to drive interspecific variation in EPP. These results suggest that although variation in extra-pair mating system can have a phylogenetic component (Arnold and Owens 2002, Griffith et al. 2002), it might also be more labile than previously thought, obscuring our understanding of the evolution of EPP (Kingma et al. 2009).

Geographic variation in EPP rates

There is considerable geographic variation in EPP rates among *Tachycineta* swallows (Figure 3.1), present between different populations of the same species (e.g., 61 to 78% nests with extra-pair young in *T. leucorrhoa*), as well as between closely related species breeding at different sites (e.g., 12 to 78% of nests with extra-pair young for the sister species *T. meyeni* and *T. leucorrhoa*, respectively, Figures 3.1 and 3.2). Geographic differences in EPP have been predicted to follow a latitudinal pattern of temperate/tropical variation (i.e. Stutchbury and Morton 1995, 2008). However, we did not detect a strong effect of latitude on this trait (Figure 3.3). Because this lack of a relation with latitude was driven by data from *T. meyeni* alone, it is important to evaluate whether that species was the exception that proved the rule or refuted it. The population of *T. meyeni* breeding in Tierra del Fuego may be an outlier to a general latitudinal trend. Other life-history traits in this population do not fit the pattern of geographic variation described in multi-species studies (i.e., small clutch size in *T. meyeni*, Liljesthröm unpl. data; Jetz et al. 2008) and many aspects of the

breeding biology of this population may be responses to the distinctively extreme climate of its Fuegian breeding site. One of the challenges of the latitudinal hypothesis is that is does not specify exactly which environmental features act proximally to cause a latitudinal pattern of variation. Further field work, concentrated on sites with extreme sets of environmental variables, could help elucidate which of the many factors that change with latitude are likely to be most important. One consequence of such further work may be that Fuegian *T. meyeni* will come to be seen as only one of many exceptions to a latitudinal trend. Indeed, it may be that studies at a few more sites will be sufficient to erase any suggestion of a general latitudinal trend. We currently do not have enough information and sites sampled to tease apart these two alternatives.

Conclusion

Comparative studies of biological traits have informed our understanding of the timing and factors involved in the diversification of life-history strategies. On the other hand, field studies have provided great insight into the importance of ecological variables as drivers of variation in life-history traits. However, an integrative explanation of variation in EPP still remains elusive. The large variation of EPP rates within the South-Central American *Tachycineta* clade, between the sister taxa *T. leucorrhoa* and *T. meyeni*, and between years in the same population of *T. bicolor* (e.g., Dunn et al. 1994), indicate that neither phylogenetic history nor geography alone can explain all variation in genetic mating systems. More comparative studies of closely related species combined with studies that span strong environmental gradients are needed to increase our understanding of the broad patterns of variation in extra-pair mating systems and of life-history traits in general.

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CHAPTER 4

THE EFFECT OF BREEDING SYNCHRONY ON EXTRA-PAIR PATERNITY ACROSS THE WIDE-RANGING *TACHYCINETA* SWALLOWS

Abstract

With the use of molecular techniques in studies of avian mating systems that started in the late 1980's, empirical work has led to the development of several hypotheses to explain variation in extra-pair paternity rates. Here we tested one of these hypotheses, known as the "female breeding synchrony hypothesis" in the swallow genus *Tachycineta*. This hypothesis, proposed by Stutchbury and Morton (1995), states that the short breeding seasons experienced by birds breeding at high latitudes lead to synchronous fertile periods during which females are better able to compare the quality of potential males, thus facilitating extra-pair copulations. We compared across species and populations of *Tachycineta* swallows to assess whether latitudinal variation in breeding synchrony is associated with interpopulation differences in extra-pair paternity rates. Although extra-pair paternity and breeding synchrony appeared to be positively correlated when considering tropical and northern species, the correlation was lost when populations from the Southern Hemisphere were added to the analysis. Synchrony was not explained by latitude nor was extra-pair paternity explained by breeding synchrony. We suggest breeding synchrony may be one of several factors acting in concert to influence the trade-offs being made by female birds during mating, and simple single-hypothesis explanations are not likely to yield reliable explanations.

Introduction

Female breeding synchrony has been proposed to explain some of the substantial variation observed in rates of extra-pair paternity (EPP) across avian species and populations (Stutchbury and Morton 1995). Under this hypothesis, high female breeding synchrony leads to high EPP rates because synchronously breeding females are better able to compare the quality of potential mating partners, facilitating their extra-pair mating decisions. Therefore, ecological factors affecting female synchrony in the breeding populations will have an indirect effect on the females' mating decisions and the rates of EPP. This association between breeding synchrony and EPP may also cause broad scale ecological variation in EPP rates, which are generally lower for species breeding in the tropics than for those breeding at higher latitudes (Stutchbury and Morton 1995, 2001, 2008). Stutchbury and Morton (1995, 2001, 2008) suggest that differences in the duration of the breeding period between temperate (short season) and tropical (long season) species dictate differences in female breeding synchrony, which in turn explain these latitudinal differences in EPP rates.

An alternative view of the relationship between synchrony and paternity suggests that higher reproductive synchrony may limit the opportunities for extra-pair matings (Birkhead and Biggins 1987, Westneat et al. 1990), leading to low rates of EPP. Males will have few opportunities to seek extra-pair copulations if they have to guard or attend their social mates during the fertile period to avoid being cuckolded, and all females in the population are fertile around the same time. While the hypothesis proposed by Stutchbury and Morton (1995) assumes that females are actively choosing and seeking extra-pair copulations, this alternative hypothesis

(Birkhead and Biggins 1987, Westeneat et al. 1990) suggests a major role of males in determining the rate of EPP in systems where mate guarding or attendance is common.

The predictions made by these two alternative hypotheses have proven to be hard to distinguish in most traditionally territorial birds: the many studies that have examined the correlation between breeding synchrony and paternity rates among individuals in the same breeding population and season have yielded conflicting results, and no clear trend (reviewed in Griffith et al. 2002 and Macedo et al. 2008). This is in part because within-season studies are weak tests for the positive relationship between synchrony and EPP, as females might be evaluating males in periods of high breeding synchrony and base later mating decisions on this prior evaluation (Stutchbury 1998a, 1998b). The best test for this hypothesis may thus be at the species level (Stutchbury and Morton 2008) and should include different populations breeding at different latitudes that would result in differences in breeding synchrony (Stutchbury 1998a). The equivocal results so far may also have been due in part to the fact that the direction of the relationship between EPP and reproductive synchrony will depend on whether extra-pair copulations are male or female driven, and on the intensity of mate attendance in the species under investigation (reviewed in Neudorf 2004). Second, the sparse sampling of tropical and southern hemisphere birds (Griffith et al. 2002, Arnold and Owens 2002, Macedo et al. 2008, Stutchbury and Morton 2008), combined with the very few groups of closely related taxa for which EPP data are currently available, limit our ability to make robust generalizations about variation in avian genetic mating systems.

Here we use comparisons among species and populations of the swallow genus *Tachycineta* to explore whether latitudinal variation in breeding synchrony is

associated with interspecific variation in EPP rates. We test for this positive relationship between breeding synchrony and paternity because in these swallows EPP rates are more likely to be driven by female mating decisions rather than male control of mating opportunities—*Tachycineta* swallows exhibit weak mate guarding (Leffelaar and Robertson 1984, Check and Robertson 1994, Beasley 1996, Moore et al. 1999, VF pers obs for two additional South American species), females go on long unattended forays during their fertile period (Dunn and Whittingham 2005, Stapleton and Robertson 2006), and they have control over their extra-pair copulations (Lifjeld and Robertson 1992). This is, to our knowledge, the first study to look at species variation in EPP rates and breeding synchrony in a phylogenetically constrained group spanning a broad latitudinal range. To generate these comparisons, we (i) gathered information on EPP rates from five *Tachycineta* species, distributed across North, Central and South America; (ii) calculated female breeding synchrony indices (Kempenaers 1993) for these same species; and (iii) tested for an association between EPP rates and breeding synchrony across species. If female breeding synchrony has a strong general effect on rates of EPP, interspecific differences in EPP rates among these five *Tachycineta* species should be positively associated with the substantial differences in the length of the breeding seasons reflected in their synchrony indices (Stutchbury and Morton 1995).

Materials and methods

Study species

We used the genus *Tachycineta* in our analysis for a number of reasons. First, and most importantly, the swallow genus *Tachycineta* exhibits large variation in genetic breeding systems (Ferretti et al. Chapter 3). Second, through the use of standardized protocols (i.e. equal cavity size, box availability, etc.; see field methods section below)

in our nest-box colonies we can control nesting conditions to a large extent, leaving fewer conflicting variables and giving us more power to measure variation in EPP rates and synchrony. Last, *Tachycineta* is a monophyletic group (Whittingham et al. 2002), has a very broad latitudinal range (Turner and Rose 1989) and is already known for its tropical/temperate variation in genetic mating system in two of its species (Moore et al. 1999). In our analysis we sampled five of the nine species in the genus: two north temperate, one tropical, and two south temperate species, giving us a wide coverage of latitudes in a single group of closely related birds.

Tachycineta swallows are typically found in open habitat near water, often close to woodland and houses (Turner and Rose 1989), and they readily use nest-boxes for breeding. We collected new data from four species—two different breeding populations of White-rumped Swallow (*T. leucorrhoa*) in Buenos Aires Province, Argentina; Chilean Swallow (*T. meyeni*) in Tierra del Fuego, Argentina; Violet-green swallow (*T. thalassina*) in California and Oregon; and Mangrove Swallow (*T. albilinea*) in Belize—and used previously published data for the fifth species, Tree Swallow (*T. bicolor*) considered in this analysis, as well as for a second population of Mangrove Swallows nesting in Panama (both from Moore et al. 1999). Details on the locations of the breeding colonies and numbers of nest-boxes for each can be found in Ferretti et al. (Chapter 3) and Moore et al. (1999).

Field methods and genetic sampling

At each of our colonies, nest-boxes are spaced at 20-35m distances and checked every other day for the length of the breeding season. During nesting we attempted to capture both adult breeders using box traps (see http://golondrinas.cornell.edu for more information on boxes, traps and sites). At this time, we marked adult birds,

banded them with aluminum bands and took a blood sample for paternity analyses. For each active nest we recorded lay date and length of the laying period. Clutch size was recorded as the maximum number of eggs found in the nest. We took blood samples (20-70µl) from nestlings when they were 7-9 days old. All blood samples—from adults and nestlings—were taken in heparinized capillary tubes via brachial venipuncture and then stored in Queens' lysis buffer (Seutin et al. 1991). When nestlings were found dead in the nest before day 7-9, we collected a sample from their pectoral muscle and stored it in 96% ethanol.

Paternity analyses

Methodological details can be found in Ferretti et al. (Chapter 1, 3). Briefly, paternity analyses were based on microsatellite's patterns of allelic variation (Makarewich et al. 2009). We ran 3-4 multiplexed PCR reactions per individual, each containing three to five primers. PCR products were then run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), and the sizes of the microsatellite alleles estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems). Allele frequencies and genetic population data (i.e. expected and observed heterozygosity) for each locus were calculated using the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007), and paternity analyses were carried out using the same program. For all species, the combined exclusion probability with one known parent (the mother) was > 0.9999.

Synchrony measures

To study the effect of breeding synchrony on extra-pair behavior among species and populations we used a measure of synchrony (synchrony index or SI) following the formula presented by Kempenaers (1993). This synchrony index represents the

average of the proportion of fertile females in the population that overlapped with each female's fertile period (Kempenaers 1993). For the calculation we used the number of fertile days for each female defined as six days prior to the laying of the first egg (Ardia et al. 2006) up to the day the penultimate egg was laid. We did not have enough data to calculate the synchrony index for the population of *T. thalassina* breeding in Oregon, so we only use this population in our analysis of latitudinal variation in EPP.

Data analyses

To explore whether interspecific differences in EPP rates could be explained by female breeding synchrony, we tested for a correlation between synchrony indices and EPP rates. In addition we looked at the latitudinal pattern of variation in synchrony by examining the correlation between the synchrony indices and the absolute latitudes of the populations sampled. All analyses were run in the statistical packages JMP 8 (SAS Institute Inc. 2009) and SPSS 14 (SPSS Inc. 2005).

Results

Extra-pair paternity rates

EPP rates for the five *Tachycineta* species show substantial variation across populations, from a low of 12% of nests having extra-pair young (and 5% of extra-pair nestlings) in *T. meyeni*, to a high of 77-89% of extra-pair nests (55-69% extra-pair nestlings) in *T. leucorrhoa*, *T. thalassina* and *T. bicolor* (Dunn et al. 1994, Barber et al. 1996, Kempenaers et al. 1999, Moore et al. 1999, Whittingham and Dunn 2001, Whittingham et al. 2006, O'Brien and Dawson 2007, Stapleton et al. 2007, Crowe et al. 2009, Dunn et al. 2009, Ferretti et al. Chapter 1, 3).

Synchrony indices

Synchrony varied widely across sites and species, as evidenced by the difference of almost an order of magnitude between the lowest percentage of overlap in breeding cycle among females in the population in *T. albilinea* and the highest in *T. thalassina* (Table 4.1). However, we did not find a significant correlation between synchrony index and absolute latitude (Pearson's r = 0.488, p = 0.266, N = 7, Figure 4.1), suggesting that synchrony might be related to other more causal factors that co-vary weakly with latitude (i.e. availability of food, photoperiod, ambient temperatures) or not (e.g. altitude).

Table 4.1 Female breeding synchrony and latitude for the seven populations of *Tachycineta* species sampled. SI: synchrony index calculated following Kempenaers (1993); N: number of nests sampled.

Species	SI (%)	N (nests)	Latitude of colony sampled	Comment
T. bicolor	47	57	44º34'N	in Moore et al. 1999
T. thalassina	70.03	19	37º58'N	
T. albilinea	7.7	48	9º10'N	in Moore et al. 1999
	17.92	55	17º36'N	
T. leucorrhoa	22.58	193	35º34'S	
	16.07	73	36º25'S	
T. meyeni	27.28	54	54º44'S	

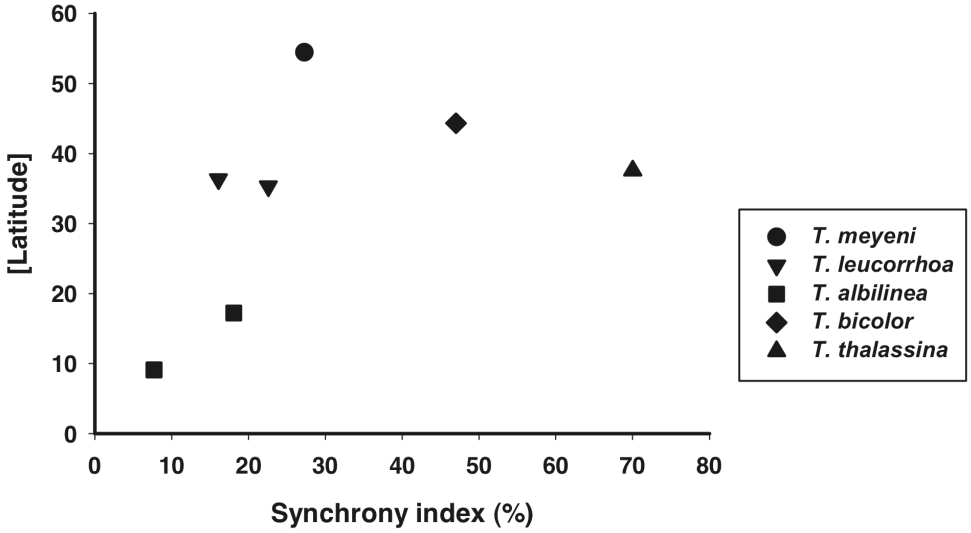

Figure 4.1 Scatter plot for latitude versus synchrony index. Latitude was considered in its absolute value; that is, irrespectively from whether the sampled colony was located north or south of the equator. Synchrony index was calculated following Kempenaer's (1993) formula. Symbols represent populations sampled. Two populations were sampled for *T. leucorrhoa* and *T. albilinea* at different latitudes.

EPP and synchrony

Despite there being considerable interspecific variation in both EPP and breeding synchrony, these two variables were not significantly correlated (Pearson's $r = 0.474$, $p = 0.282$, $N = 7$, Figures 4.2 and 4.3).

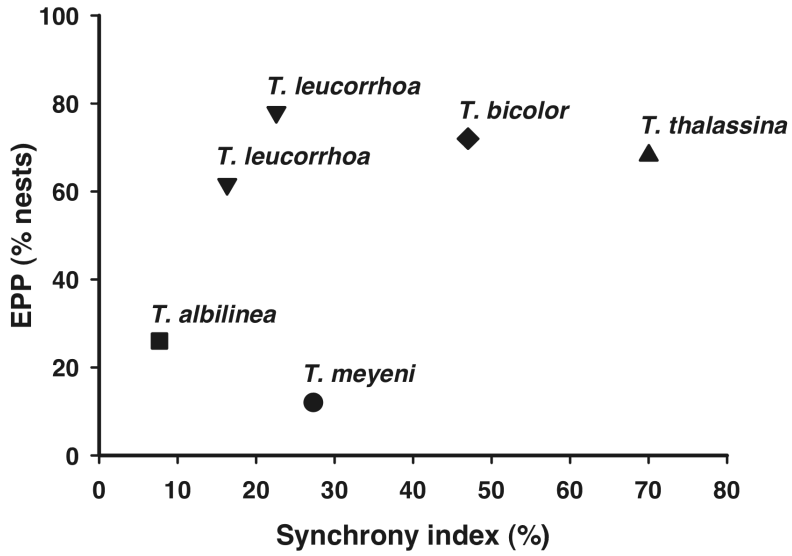

Figure 4.2 Scatter plot for EPP rates versus synchrony index. EPP rates are presented here as the proportion of nests in the population that had extra-pair young. Synchrony index was calculated following Kempenaer's (1993) formula. Symbols represent populations sampled. Two populations are represented here for the species *T. leucorrhoa.*

Discussion

We tested the female breeding synchrony hypothesis by examining interspecific differences in EPP rates and breeding synchrony in five species in the genus *Tachycineta*. The populations of the five species used in our analysis breed at markedly different latitudes, from high northern and southern latitudes throughout the tropics, and show extensive variation in the rates of EPP (Ferretti et al. Chapter 3). Our results suggest that differences in EPP rates among species of *Tachycineta* are not explained by differences in their breeding synchrony (Figures 4.2 and 4.3). Moreover, differences in the length of the breeding season, and the correspondingly high

variation in their breeding synchrony, are not related to latitude (Table 4.1, Figures 4.1 and 4.3). We were, however, expecting different results. In a previous study, Moore and co-authors (1999) compared the north temperate Tree Swallow (*T. bicolor*) with the tropical Mangrove Swallow (*T. albilinea*) and found that differences in EPP rates and synchrony followed the direction predicted by the breeding synchrony hypothesis (Stutchbury and Morton 1995, Moore et al. 1999). They suggested that the longer breeding season in the tropics resulted in a lower degree of overlap among breeding birds, and thus lower breeding synchrony, which in turn resulted in lower rates of EPP (Stutchbury and Morton 1995, 2008, Stutchbury 1998a, 1998b, Moore et al. 1999). But when we included more related species in this comparison we found that breeding synchrony does not seem to explain variation in EPP rates and synchrony does not follow a latitudinal pattern of variation.

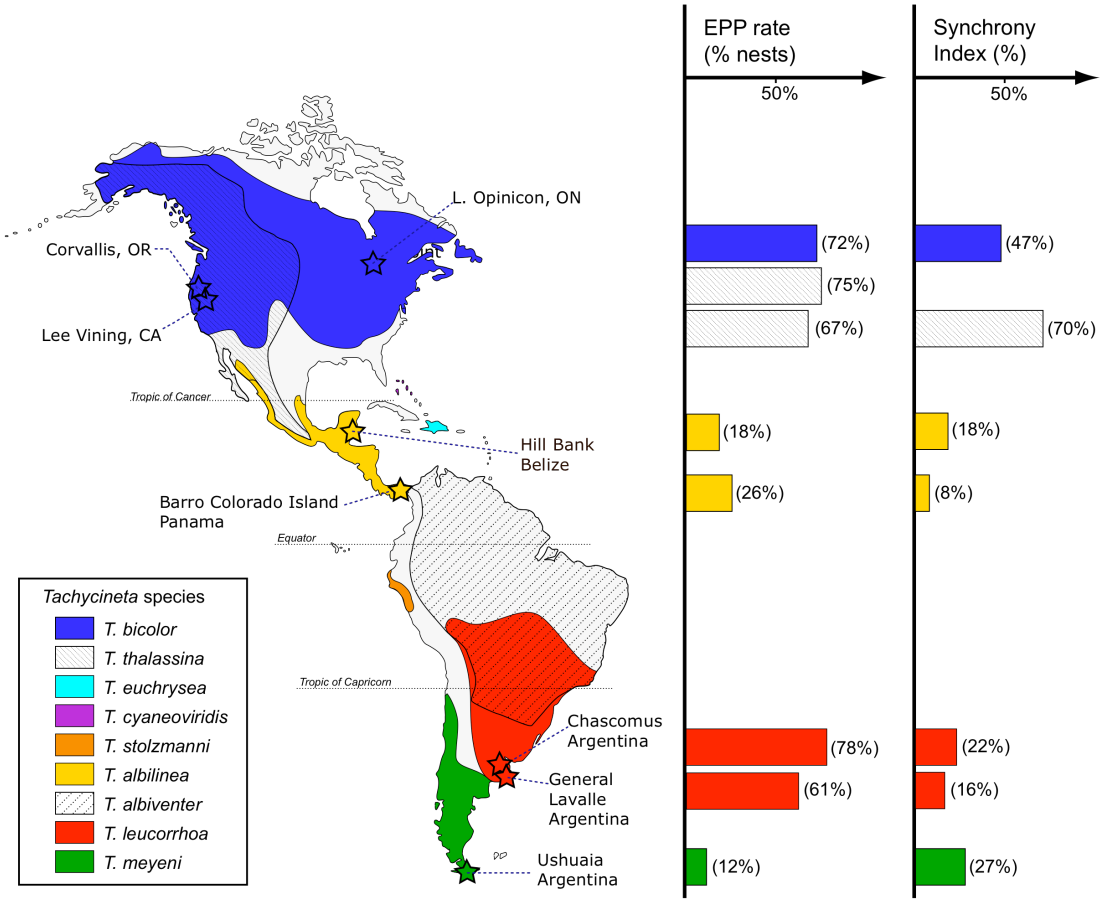

Figure 4.3 Geographic distribution of *Tachycineta* swallows and locations of breeding colonies sampled in this study. On the right, bars represent % of nests in the population with extra-pair young (EPP rate) and % of overlap among breeding birds (synchrony index) for all the populations sampled.

Since Stutchbury and Morton (1995) first proposed the female breeding synchrony hypothesis, support for it has been mixed. Some of the strongest challenges to it have noted the paucity of tropical and southern species data and the conflicting results of the many empirical studies of within population variation in synchrony and EPP rates (see Griffith et al. 2002 for a review of these studies). One of the caveats we see in all of these analyses, however, is that there are no closely related groups that have been included in these studies, that span northern, tropical and southern latitudes (Arnold and Owens 2002 Appendix A), and the tropical and southern species surveyed to date still represent an absurdly low percentage of the total species inhabiting these biogeographical realms (i.e. <0.1% for tropical species Macedo et al. 2008). By focusing on variation in one genus of ample distribution we were able to look at the relationship proposed by the breeding synchrony hypothesis from a different angle.

Our work reminds us of the importance of comparative studies that span different latitudes and different life-history strategies. The addition of related species and populations of broad distribution revealed a different pattern that the one expected, and broke the seeming correlation between breeding synchrony and paternity when only two species were used for comparison (Moore et al. 1999). Had we not added these other representatives, we would have been left with a spurious result. This is not to say that current ecological factors such as breeding synchrony are not playing an important role in determining the behavior of birds and influencing the evolution of variation in mating systems; in fact we believe they might. But we need to have a closer look at the relationships among the different variables and life-history traits. Synchrony should be related to seasonality, but seasonality does not always vary with latitude. Seasonality is related to a combination of weather patterns, dependent on the geography of the area surveyed (i.e. altitude, continentality and/or distance to the

ocean), that in turn might have an effect on other life-history traits such as survival and clutch size (Martin 2004). Life-history traits are interconnected by a complex array of relationships (Winkler 2000) and the resulting life-history strategies are believed to be adaptive outcomes of the trade-offs among the different traits and the selective nature of different ecological variables (see Figures 2 and 3 in Martin 2004). Isolating one trait and one ecological variable will not provide further enlightenment in life-history theory or the evolution of avian mating systems (Westneat and Stewart 2003).

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CHAPTER 5

EXTRA-PAIR PATERNITY IN BIRDS: A LIFE-HISTORY DILEMMA?

Since 1987 when DNA molecular techniques were first applied to behavioral and ecological studies of mating systems in birds (Burke and Bruford 1987), the long-standing assumption that 90% of avian taxa are monogamous (Lack 1968) has steadily become less tenable. Now, it is difficult to speak of avian mating systems without distinguishing between social and genetic mating systems. While genetic monogamy refers to the association of a male and a female for breeding purposes that results in the exclusive parentage by the breeding pair, social monogamy accompanied by genetic polygamy refers to a situation in which a male and a female breed and raise young together but some, or all, of the offspring in the nest are sired by other adults in the population as a result of a mixed reproductive strategy by the social parents.

The first studies that came out on this subject applied molecular techniques to the study of wild bird populations to evaluate the incidence of mixed reproductive strategies by males and females (e.g. Quinn et al. 1987). We have now come to a point where, instead of asking *what* is the incidence of extra-pair paternity (EPP), research questions are focused on *why* there is variation in this incidence both within and between populations and species (Bennett and Owens 2002, Griffith et al. 2002, Westneat and Stewart 2003). This shift resulted in the proposal by several authors of different factors that could account for the observed variation in genetic mating systems and the publication of at least three major review papers in the last eight years (Griffith et al. 2002, Westneat and Stewart 2003, Neudorf 2004). Despite all the attention received and some careful comparative analyses (e.g. Arnold and Owens

2002) there still does not seem to be a unified understanding of the causes of variation in EPP.

What are we still missing? Lack of unified understanding may be due to 1) insufficient sampling of EPP rates for taxa with certain life-histories (e.g., tropical, south- temperate taxa) and across groups of recent divergence (below the taxonomic level of Family), and/or 2) inadequate understanding of the links between EPP and other life-history traits. I review these topics below.

Insufficient Sampling

All of the data available on paternity rates in birds today represent a very small minority of bird species in the world (Neudorf 2004). With most bird diversity concentrated in the tropics and the southern hemisphere (Hawkins et al. 2003) and almost all studies performed in northern hemisphere birds, it should come as no surprise that, as we start looking at non-northern populations in more detail the patterns of variation in mating systems become unclear, making it almost impossible to predict extra-pair mating strategies. This is what has probably contributed to the plethora of discussion forums, contradicting responses to papers, and heated arguments among members of the ornithological community (e.g. Stutchbury 1998a, 1998b, Weatherhead and Yezerinac 1998, Westneat and Stewart 2003, Akçay and Roughgarden 2007, Macedo et al. 2008, just to name a few). If we want to gain a global understanding of mating systems we need to gather globally distributed data (Stutchbury and Morton 2001, Neudorf 2004). The effect of sampling bias on the rejection or support of hypotheses that explain variation in EPP rates was made clear in Chapters 3 and 4 of this Dissertation—the analysis of variation among closely related Tachycineta species allowed us to simultaneously evaluate diversification in

EPP rates from a historical and a contemporary ecological standpoint, contradicting the results from previous studies when only groups of older divergence were used (Arnold and Owens 2002, Bennett and Owens 2002). For example, in their review Macedo et al. (2008) point out that only ~ < 0.1% of the tropical species have been sampled for paternity studies. With such low numbers, generalizations about the different factors that might affect geographic variation in EPP are deemed to fail. Similarly, in Arnold and Owens' (2002) and Bennett and Owens' (2002) analyses they were able to use studies describing only 88 species of birds—of the nearly 10,000 extant species—from 36 different taxonomic Families, where only two families had more than two species in the same genus (*Falco* and *Parus*) for which there was paternity information; and all of these were on populations inhabiting Europe and/or North America. This is mainly due to the scarcity of studies south of the equator, and the paucity of data on closely related groups, both of which obstruct the interpretation of historical data. In sum, we don't understand *why* there is variation in EPP rates because we have a biased sample of taxa and thus inadequate sampling of the ecological/evolutionary factors that may have shaped, and still be shaping, EPP rates. So the shift from asking *what* EPP rates are to asking *why* they are variable may be premature.

Understanding links between EPP and other life history traits

However, unbiased sampling might not be the only improvement to aspire in our studies. Mate choice, both genetic and social, can be directly affected by selection acting on other life-history traits. Historically, research on the ecology of avian life-histories had focused on intra-individual trade-offs in life-history traits, with much less attention to inter-generational trade-offs (Stearns 1992). These inter-generational trade-offs come into play when the adults' evolutionary outcome, in terms of their life-

history traits, affects their offspring's traits. For example, one trade-off adult birds face is how much of the available reproductive energy to invest in number of eggs produced (i.e. clutch size) versus producing fewer but larger eggs. In this case, larger eggs might produce larger offspring that will have a better chance of survival (Winkler and Wallin 1987). Then, what was considered only to be an intra-individual trade-off becomes an inter-generational trade-off. Additionally, intra-individual and inter-generational traits can interact with each other, particularly at the level of sexual conflict and reproductive allocation. Such is the case with the interaction between parent-offspring conflict, sibling rivalry and siblicide (Mock 1984). For example, in Cattle Egrets eggs hatch asynchronously giving the early hatchers an advantage over late hatchers. In most years food is relatively scarce and the larger-older siblings will kill the smaller-younger ones. However, in years of high food abundance all chicks might survive (Mock and Ploger 1987). Therefore, sibling competition and siblicide drive parents to direct resources only to those offspring that have a greater chance for survival, while enabling them to keep their reproductive costs low. Individuals will thus be faced with ecological and evolutionary scenarios that may affect their fitness in different ways: how can offspring number and survival be maximized?

EPP is among the most important of these inter-generational trade-offs, affecting not only sibling competition and survival, but also parental care, and resource allocation by the breeding pair. I believe it is possible to gain a better understanding of the interplay of EPP with other life-history variables (i.e. parental care, survival) and how they relate to each other by exploring the co-variation of these traits both together and independently.

Hypothesized relationships between extra-pair paternity and other life-history traits

Understanding variation in life-history traits in birds has been an active area of research since the 1940's (reviewed in Ricklefs 2000), but the evolutionary and ecological relationships, as well as the physiological and developmental relationships, among some life-history traits are still poorly understood (Martin 2004, Macedo et al. 2008).

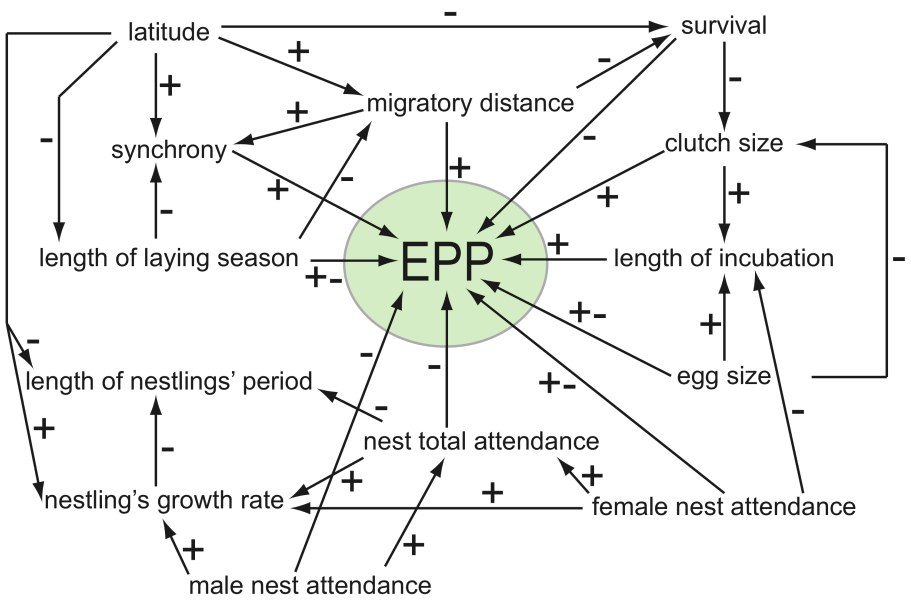

Figure 5.1 Hypothesized relationships among life-history and other ecological traits. The sign and direction of the arrows represent the expected relationship between the trait where the arrow starts and the trait where it ends.

Different species, even those closely related, exhibit substantial variation in their life cycles (as seen in Chapters 1through 4 of this Dissertation). Some species migrate and some are year-round residents, some roost in the same places where they

breed, some form long term partnerships; and there is variation in the rates of within- and extra-pair copulations, variation in the number of eggs they lay, and in the length of each of these periods. When we parameterize all this information we end with a complex network of interactions among traits (Figure 5.1).

For example, adult survival can have an effect on the duration of the pair bond and can impact the reproductive share that the adults put into each breeding attempt, which has the potential of affecting parental care tactics, and thus, mating systems (i.e. the degree of extra-pair paternity). Survival may in turn be influenced by migratory behavior and migratory distance, a factor that will likely be related to latitude. Similarly, male nest attendance should be inversely related to EPP at the nest, and increased attendance by both adults should have a positive effect on the rate of growth of nestlings. These are just two examples of a much larger and complex network of interactions and trade-offs. Life-history traits respond to many forces; it is naïve and too simplistic to expect to find high explanatory power for the variation in EPP based on one, or a few, parameters of this complex network. Comprehensive studies on the relationships in this network can shed some light on the main drivers of variation in mating systems—future studies of variation in traits such as EPP will require integrating across different levels and a detailed understanding of life history traits and their population-level and evolutionary drivers.

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