

CONTEXT-DEPENDENT FITNESS CONSEQUENCES OF EXTRA-PAIR PATERNITY IN
TREE SWALLOWS (TACHYGINETA BICOLOR)

A Dissertation

Presented to the Faculty of the Graduate School
of Cornell University

In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by

Kelly Kristen Hallinger

January 2017

© 2017 Kelly Kristen Hallinger

CONTEXT-DEPENDENT FITNESS CONSEQUENCES OF EXTRA-PAIR PATERNITY IN
TREE SWALLOWS (*TACHYGINETA BICOLOR*)

Kelly Kristen Hallinger, Ph.D.

Cornell University 2017

Extra-pair paternity (EPP) – the siring of offspring by a male other than a female’s social partner – is a widespread phenomenon in birds. Researchers have long hypothesized that EPP must confer a genetic advantage to extra-pair offspring (EPO) over their maternal half-siblings (within-pair offspring: WPO), but support for this hypothesis has been definitively mixed. This lack of consensus might have arisen because fitness differences between EPO and WPO are only apparent in some environments. Here, I examine the role that environmental context plays in shaping the relative fitness of Tree Swallows (*Tachycineta bicolor*) of differing paternity status. In Chapter 1, I manipulated perceived predation risk in a population of breeding adults and monitored survival, growth, and glucocorticoid stress reactivity of their nestlings. I used an alternating block design, in which the treatment applied to each nest box was reversed in successive years of study. In so doing, I discovered that EPO were larger, longer-winged, and heavier than WPO, but only in nests that had been exposed to predators. In the absence of predators, WPO were heavier than EPO, a result that was not predicted by any existing hypothesis that seeks to explain female extra-pair behavior in birds. In Chapter 2, I took advantage of historical banding and nest records, as well as archival DNA, to investigate whether environmental conditions during development influenced the relative probability of recruitment of EPO and WPO into their natal population. I was able to identify several early-life variables that impacted the likelihood of subsequent recruitment, but found no differences in recruitment between EPO and WPO under any set of early-life circumstances. In Chapter 3, I examined long-

term fitness outcomes of female swallows of known paternity status who had been born on our site and recruited into the population as adults. I found that WPO survived longer and produced more fledglings than EPO, and that their superior performance was attributable to high developmental plasticity that enabled individuals reared in particularly benign environments to outperform less plastic EPO. Collectively, these findings suggest that EPO and WPO, rather than being of intrinsically different quality, as has often been assumed, may represent alternative reproductive strategies, each of which performs best in certain environments.

BIOGRAPHICAL SKETCH

Kelly K. Hallinger received her B.S. in Biology in 2009 from the College of William and Mary, where she spent three years studying the effects of mercury contamination on songbirds under the supervision of Dr. Daniel Cristol. Her undergraduate Honors thesis examined the effects of mercury on adult survival of Tree Swallows nesting along a contaminated river in Virginia.

In 2009, Kelly began graduate studies at Cornell University under the direction of Dr. David Winkler. Her dissertation investigated the role of environmental variation in shaping fitness outcomes of Tree Swallows sired by within-pair and extra-pair males. She defended her dissertation in August 2016, and has since begun a postdoctoral research position at the University of Arizona with Dr. Renée Duckworth. There, Kelly is studying how patterns of hybridization between Western and Mountain Bluebirds vary with ecological succession.

To my family and friends, who made all this possible

ACKNOWLEDGMENTS

This dissertation represents the culmination of many years of effort and hard work by a great number of people. First, I would like to thank my advisor, Dr. David Winkler, for his unceasing encouragement and support. It is one thing to supervise a student who is doing well, but it is something else entirely to help a student who is struggling. Wink recognized early on that the desire to be a scientist had to come from me, but made it clear that no matter my professional aspirations, what he wanted more than anything was for me to be happy. The dual independence and support that he afforded me was critical to my eventual success.

I would also like to thank my committee members, Dr. Irby Lovette, Dr. Michael Webster, and Dr. Maren Vitousek, for their guidance and advice throughout this process. I could not have asked for a more thoughtful and engaged committee, and I was very fortunate in their willingness to oversee my progress. In particular, I owe Irby a debt of gratitude for welcoming me into the Fuller Evolutionary Biology Lab, where I carried out all of my genetic work. Maren provided immeasurable intellectual, logistical, and financial support for my field study examining interactions between predation risk and extra-pair paternity, and deserves special acknowledgement for her role in this project.

Field work for this study was carried out over a number of years by several generations of students and researchers who have long since left Cornell. The long-term record of Tree Swallow breeding ecology that is their legacy was a critical part of my dissertation. Long-term field studies are invaluable for asking questions about ecology and evolution, but often outlive any single graduate student's career. I really did stand on the shoulders of giants.

Genetic work for this dissertation was carried out in the Fuller Evolutionary Biology Lab at the Cornell Lab of Ornithology, where I benefitted greatly from the training and expertise of Laura Stenzler, Amanda Talaba, and Dr. Bronwyn Butcher.

All told, I had three labs in which I made my home. The Winkler, Vitousek, and Fuller labs were wonderful sources of advice and inspiration, and I cannot possibly quantify the value that they added to my graduate career. In particular, I would like to thank Amos Belmaker, Anna

Forsman, Justin Proctor, and Ryan Shipley for being such a wonderful core group of graduate students of which I was privileged to be a member.

Throughout my time at Cornell, I was humbled by the willingness of so many people to come to my assistance in ways both small and large. It is truly difficult for me to think of a single individual that I interacted with who did not enhance my professional or personal development in some way. Several are deserving of special mention: Dr. Erika Mudrak at the Cornell University Statistical Consulting Unit provided statistical advice throughout my graduate career. Dr. Kelly Zamudio and Dr. Monica Geber offered a wonderful course on grant writing that will assist my writing process for many years to come. Kelly and Dr. Liz Balko offered me several invaluable teaching opportunities. The administrative support staff in the Department of Ecology and Evolutionary Biology (DeeDee Albertsman, Carol Damm, John Howell, Patty Jordan, LuAnne Kenjerska, Brian Mlodzinski, Timothy Larkin, and Jennifer Robinson), and at the Lab of Ornithology (Cindy Marquis, Jeff Payne, and Sue Taggert) were instrumental in helping me navigate the bureaucratic intricacies of graduate school.

This dissertation was principally funded by grants from the Athena Fund at the Cornell Lab of Ornithology, the Andrew W. Mellon Foundation, and the Paul P. Feeny Student Research Fund at Cornell University. Additional support was provided by National Science Foundation grants to David Winkler (DEB-0717021 and DEB-1242573) and Maren Vitousek (IOS-1457251).

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH	iii
DEDICATION	iv
ACKNOWLEDGMENTS	v
TABLE OF CONTENTS.....	vii
LIST OF FIGURES	ix
LIST OF TABLES	x

CHAPTER 1: PERCEIVED PREDATION RISK MODULATES RELATIVE GROWTH OF EXTRA-PAIR AND WITHIN-PAIR TREE SWALLOWS (*Tachycineta bicolor*)

Abstract	1
Introduction.....	1
Methods.....	4
Study Species	4
Fitness Metrics	5
Field Site	6
Predator Manipulations	7
Nest Monitoring	8
Capture and Sampling	8
Behavioral Observations	9
Parentage Analysis	9
Hormone Analysis.....	10
Statistical Analyses	10
Results.....	12
Paternity Analysis	12
Nestling Fitness Outcomes.....	13
Sire Characteristics.....	15
Females Characteristics.....	16
Parental Feeding Rates	16
Discussion.....	17

CHAPTER 2: EXTRA-PAIR PATERNITY DOES NOT INFLUENCE LOCAL RECRUITMENT OF FLEDGLING TREE SWALLOWS (*Tachycineta bicolor*)

Abstract	25
Introduction.....	26
Methods.....	30
Study Species	30
Study Sites.....	31
Field Methods.....	31
Sample Selection.....	32

Genetic Analysis	34
Statistical Analyses	35
Results.....	40
Factors Predicting Recruitment.....	40
Paternity Analysis	41
Genetic Benefits	42
Discussion.....	45

**CHAPTER 3: DIFFERENCES IN DEVELOPMENTAL PLASTICITY INFLUENCE
LIFETIME REPRODUCTIVE SUCCESS OF WITHIN-PAIR AND EXTRA-PAIR TREE
SWALLOWS (*Tachycineta bicolor*)**

Abstract.....	49
Introduction.....	50
Methods.....	53
Study Species	53
Study Sites.....	53
Sample Selection.....	54
Paternity Analysis	54
Fitness Metrics	55
Environmental Metrics.....	58
Statistical Analyses	62
Results.....	64
Dispersal.....	64
Annual Reproductive Success.....	65
Lifespan.....	69
Lifetime Reproductive Success.....	71
Discussion.....	73
APPENDIX.....	82
BIBLIOGRAPHY.....	93

LIST OF FIGURES

1.1 Schematic layout of study site, showing treatment groups	7
1.2 Day 12 mass of nestling EPO and WPO reared in the presence or absence of predators	15
1.3 Day 12 mass of nestling EPO and WPO on each side of study site	21
2.1 Schematic representation of alternative genetic benefits hypotheses	40
3.1 Interactive effects of extra-pair status and environment on fledgling production	68
3.2 Interactive effects of extra-pair status and environment on minimum lifespan.....	70
3.3 Interactive effects of extra-pair status and environment on lifetime reproductive success	73

LIST OF TABLES

1.1	Nine loci used to assess parentage of Tree Swallows.....	13
1.2	Summary statistics for best-supported models of nestling fitness.....	14
1.3	Summary statistics for best-supported models of sire characteristics.....	16
2.1	Predictions made by each variant of the genetic benefits hypothesis.....	30
2.2	Variables included in most parameterized model investigating effects of early-life environment on recruitment.....	36
2.3	GLMM results detailing how early-life environment influences recruitment.....	41
2.4	Eight loci used to assess parentage of Tree Swallows.....	42
2.5	Model selection results for models evaluating whether genetic benefits of EPP influence recruitment.....	43
2.6	Model selection results for models evaluating whether genetic benefits of EPP influence recruitment, separated by sex.....	44
3.1	Environmental metrics used in models of recruit life history.....	61
3.2	Model selection results for natal and breeding dispersal distance.....	65
3.3	Model selection results for annual reproductive success of female recruits.....	67
3.4	Model selection results for minimum lifespan of female recruits.....	70
3.5	Model selection results for lifetime reproductive success of female recruits.....	72
3.6	Significant pairwise differences between EPO and WPO life history.....	76

CHAPTER 1

PERCEIVED PREDATION RISK MODULATES RELATIVE GROWTH OF EXTRA-PAIR AND WITHIN-PAIR TREE SWALLOWS (*Tachycineta bicolor*)

Abstract

Extra-pair paternity (EPP) – the siring of offspring by a male other than a female’s social partner – is a widespread phenomenon in birds. Researchers have long hypothesized that EPP must confer a fitness advantage to extra-pair offspring (EPO), but empirical support for this hypothesis is definitively mixed. Some studies show strong evidence of genetic benefits of EPP, while an equal number show no support for this hypothesis. Though largely unexplored in the extra-pair literature, environmental context offers a remarkably simple explanation: perhaps genetic benefits of EPP are only observed in some studies because they only exist in some environments. From 2013-2015, we manipulated predator density in a population of Tree Swallows (*Tachycineta bicolor*) breeding in New York to see whether fitness outcomes of extra-pair and within-pair offspring (WPO) varied along a predation risk gradient. We used an alternating block design, in which the treatment applied to each nest box was reversed in successive years of study. Consistent with the genetic benefits hypothesis, we discovered that EPO were larger, longer-winged, and heavier than WPO in nests that had been exposed to predators. However, we also found that WPO were heavier than EPO in non-predator nests, a result that was not predicted by any existing hypothesis that seeks to explain female extra-pair behavior in birds. Our study is the first to document a reversal in relative fitness of EPO and WPO across an environmental gradient, and suggests that EPO and WPO may each confer a fitness advantage in different circumstances.

Introduction

Extra-pair paternity – the siring of offspring by a male other than a female’s social partner - occurs in up to 90% of all bird species (Griffith et al. 2002). In spite of its high

prevalence, however, its causes and consequences remain poorly understood. Males stand to benefit tremendously from pursuing extra-pair copulations, as each new partner provides an opportunity to father additional offspring. For females, however, the benefits are less clear. And yet, females appear to tolerate and, in many cases, actively solicit extra-pair encounters (e.g. Kempenaers et al. 1992, Double and Cockburn 2000).

Researchers have generated an impressive number and diversity of hypotheses to account for female extra-pair behavior, among them fertility insurance (Griffith 2007, Forbes 2014, Schmoll and Kleven 2016), genetic constraint (Westneat and Stewart 2003, Forstmeier et al. 2014, Hsu et al. 2014), and direct material gain (Townsend et al. 2010). But by far the most frequently and intensively investigated hypotheses posit that by mating outside the pair bond, females are acquiring ‘good genes’ for their offspring. Good genes can arise because females select extra-pair males that are genetically superior to their social mate (Jennions and Petrie 2000, Neff and Pitcher 2005, Richardson et al. 2005), or because females select extra-pair sires that are genetically complementary, such that offspring inherit a greater diversity of alleles (Zeh and Zeh 1996, 1997, Blomqvist et al. 2002, Arct et al. 2015). In either case, extra-pair offspring (EPO) are expected to have higher fitness than their maternal half-siblings (within-pair offspring: WPO). This prediction has been tested hundreds of times in dozens of species, and yet has been met in less than 50% of published studies (Akçay and Roughgarden 2007).

Environmental context may provide a way forward (Schmoll 2011). Evolutionary biologists have long recognized the capacity for environmental conditions to modify the expression of inherited traits. Indeed, this basic dependence of genes on environment is a cornerstone of modern biology. There may well be genetic benefits conferred to EPO, but they may not be equally expressed under all scenarios. For example, EPO may acquire genes for resistance to a novel pathogen, but these genes will only confer higher fitness in an environment in which that pathogen is present. In an environment without this pathogen, there will be no apparent difference in fitness between EPO and WPO. This same logic can be applied to any number of inherited traits in concert with the appropriate environmental context. The elegance of

this hypothesis lies in its potential to explain seemingly contradictory studies. Why might one study show strong evidence of genetic benefits and another show no such support, even when carried out in the same species? Perhaps environmental conditions differed in a way that tended to enhance or mask fitness differences between EPO and WPO (reviewed in Schmolz 2011).

This idea has only formally been tested a handful of times, but these early studies have proved promising (Schmolz 2011). In Coal Tits (*Parus ater*), EPO were more likely to recruit into the breeding population than WPO, but only if they had been born late in the season when overall breeding performance had declined (Schmolz et al. 2005). Likewise, Common Yellowthroat (*Geothlypis trichas*) EPO exhibited a stronger T-cell mediated immune response than WPO only in the colder of two study years (Garvin et al. 2006). Arct et al. (2013) experimentally stressed nestling Blue Tits (*Cyanistes caeruleus*) by brood enlargement and found that EPO exhibited a stronger humoral immune response than WPO under these conditions. In all of these examples, the suggestion is that EPO may be better equipped to manage and mitigate stress. Thus, the logic follows that fitness differences between WPO and EPO may most readily be observed when environmental conditions are sufficiently challenging (but see O'Brien and Dawson 2007).

Predation presents one such challenge. Because it is a common and potentially devastating part of an organism's environment, predation can lead to strong selection on a number of diverse organismal traits (e.g. behavior: Steinberg et al. 2014; physiology: Mateo 2007; life history: Spitze 1992). On the one hand, this makes studying prey biology difficult, as we might expect a complex and multi-tiered suite of adaptations to arise. On the other hand, the cost of being preyed upon is sufficiently high that we expect organisms to exhibit a powerful response to this stressor. If EPO are better than WPO at managing and mitigating stress, this difference should become apparent under high predation risk.

It is interesting to note fitness differences between EPO and WPO in specific environmental contexts, but it is also important to understand how such differences might arise. Here again, predation presents a complex suite of possibilities. For example, if heightened

predation risk dampens sexual signaling in prey (as in Steinberg et al. 2014), then females may be more limited in mate choice in high-predation environments. If signal dampening is uneven in the population of males (i.e. if dampening only occurs in a subset of males), then the composition of available extra-pair mates, and consequently, the genetic composition of extra-pair offspring, might change. Likewise, if adults become more risk-averse in high-predator environments and consequently modify their provisioning behavior (as in Tilgar et al. 2011), then nestling competition might change in a way that favors nestlings of one paternity status over the other. This is especially true if EPO exhibit different personalities (e.g. are more aggressive or bold) or size (e.g. are larger). These are but two possibilities. In spite of this potential for interactions between predation risk and EPP, to our knowledge, only three studies have explicitly examined avian extra-pair behavior in this context, and even then, only as it relates to extra-pair fertilization rates (Gissing et al. 1998, Bouwman 2005, Yuta and Koizumi 2015). No studies, however, have examined the relative fitness of nestlings of differing paternity status reared under experimentally heightened risk of predation.

In this study, we compare the relative fitness of extra-pair and within-pair nestling Tree Swallows (*Tachycineta bicolor*) reared under experimentally elevated or natural predation risk. We examine three metrics of nestling fitness which might be impacted by increased exposure to predators: growth, glucocorticoid stress reactivity, and survival to fledging. In so doing, we test the hypothesis that EPO will exhibit higher fitness than WPO when environmental conditions are stressful, but not when conditions are more benign.

Methods

Study Species. Tree Swallows are migratory aerial insectivores that breed throughout much of North America (Winkler et al. 2011). They are secondary cavity nesters, and will readily accept artificial nest boxes. Except for in the immediate vicinity of the nest, Tree Swallows are generally non-territorial, preferring instead to forage freely wherever food is available, and to roost (especially early in the breeding season) in large flocks consisting of

thousands of birds (Winkler et al. 2011). This is noteworthy for two reasons. First, their gregariousness has the potential to influence how they perceive and respond to predators, since predators encountered outside of the immediate “territory” are likely to be a threat. Additionally, their sociality may influence their extra-pair behavior. Indeed, Tree Swallows have one of the highest rates of extra-pair paternity of any songbird ever studied, with up to 50% of nestlings in 90% of nests being EPO (Winkler et al. 2011). Their extra-pair behavior has thus come under significant scrutiny, but as in many other species, support for good genes hypotheses has been decidedly mixed (reviewed in Winkler et al. 2011).

Fitness Metrics. We used three metrics to evaluate nestling fitness. First, we measured survival to fledging. Like many short-lived songbirds, Tree Swallows experience two major survival bottlenecks over the course of their lives. The first comes in the first few weeks of life between the time an egg is laid and the time the resultant nestling leaves its nest box. In our population, over 40% of eggs do not produce fledged nestlings (K. K. Hallinger, unpublished data). If EPO and WPO fledge at different rates under high predation stress, this difference could contribute significantly to lowered lifetime fitness.

Even if EPO and WPO fledge at equal rates, they may not fledge in equal condition. A swallow that leaves its nest box successfully can expect to encounter a series of serious challenges shortly after fledging. While still perfecting their flight abilities, fledglings must successfully acquire prey, avoid predators, exposure, and disease, undergo a full pre-basic molt, and perform a migration of more than 2000 km each way. Tree Swallows are one of the earliest migratory species to return to their breeding sites in the spring, and must often endure harsh early spring weather upon arrival there. These challenges routinely result in the deaths of many adults, but for hatch year birds, mortality may be as high as 75% (Winkler et al. 2011).

Nestlings that leave the nest in poor condition may therefore face an especially high risk of post-fledging mortality. To gauge nestling condition, we measured three size metrics (head-bill length, wing length, and mass) on days 6 and 12 post hatching. In this population, nestling

mass is a strong predictor of subsequent recruitment, with larger nestlings being more likely to return as adults (this dissertation, Chapter 2).

In addition to nestling growth, we measured both baseline and stress-induced corticosterone of Day 12 nestlings. Corticosterone is the primary avian glucocorticoid and is responsible for facilitating a suite of coordinated physiological responses to stress. These include mobilization of glucose, metabolism of fat, protein, and carbohydrates, and suppression of the immune system, growth, and reproductive behavior (Sapolsky et al. 2000, Wingfield and Romero 2001, Angelier et al. 2009). In concert, this coordinated response helps an organism prioritize and meet the energetic demands necessary for overcoming a threat to survival. The ability to efficiently upregulate corticosterone in response to a stressful stimulus and also to return to homeostasis after that threat has passed is an important predictor of fitness (Wingfield et al. 1998, Sapolsky et al. 2000). However, individuals who exhibit chronically elevated corticosterone, either because the stressor or the stress response is frequent or prolonged, may be expected to suffer fitness costs (Dickens and Romero 2013). We predicted therefore that nestlings in the best condition would exhibit low levels of circulating baseline corticosterone with strong spikes in response to acute capture stress.

Field Site. We studied a population of Tree Swallows breeding in Tompkins County, New York, USA (42.45°N, 76.37°W). The study site consisted of 46 nest boxes placed on either side of a gravel road and spaced approximately 20 m apart. Because Tree Swallows defend only a small area in the vicinity of their nest site and will communally mob intruding predators, we applied predator and non-predator treatments to opposite sides of the study site (as opposed to alternating predator and non-predator boxes or assigning treatments randomly). A total of 21 boxes each were designated as belonging to the predator and non-predator treatments. The remaining four boxes were situated between the predator and non-predator sides and served as a spatial buffer between the two treatments (Figure 1.1). Predator and non-predator sides were reversed in successive study years (2013-2015) in order to minimize any microhabitat-related

settlement preferences or fitness outcomes.

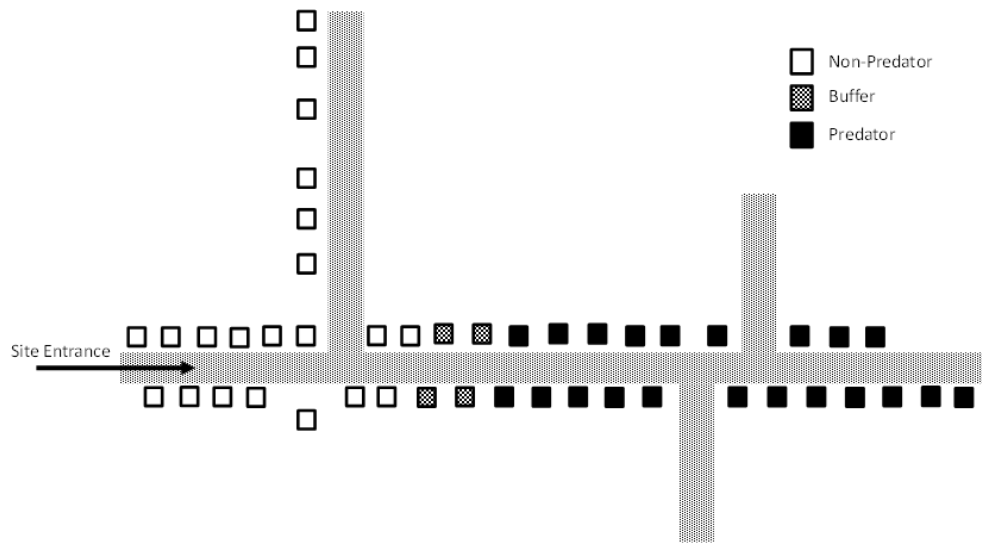


Figure 1.1: Schematic layout of study site. Each square represents a single nest box, colored to reflect its predator treatment in 2013. In 2014, predator and non-predator boxes were reversed. In 2015, treatments were reversed again, such that they were the same as in 2013.

Predator Manipulations. Nest boxes were monitored for evidence of breeding activity beginning in early April of each year. In order to reduce the chance of the experimental treatment influencing settlement, we waited to begin the manipulation until nesting material had appeared in ~50% of nest boxes. The first day of treatment was 27 April in 2013, 5 May in 2014, and 3 May in 2015. Because the goal of the predator treatment was to generate moderate chronic stress without severely disrupting breeding, we elected to perform trials three times per day on each of four days per week. Time of day and day of week were randomized as much as was possible, with the exception that treatments were never applied immediately prior to corticosterone sampling (see below).

During a single trial, we sequentially placed one of three mock predators (a snake, a cat, or a raptor) approximately three meters away from each of three randomly selected nest boxes. Each predator was mounted on a small wooden platform affixed to a 4-ft step-in fence post. This

same protocol was followed simultaneously on the non-predator side, with the important difference that three mock non-predators matched for size and taxonomy (a turtle, a rabbit, or a duck) were used to control for disturbance. Each trial lasted fifteen minutes from the time that the sixth mount was in place (typical time to put out all mounts ~6-8 minutes), and the order in which mounts were put out and collected alternated between the predator and non-predator sides in successive trials.

This treatment regime was followed until the first nestlings on the site reached eight days of age, at which point the manipulation was discontinued (7 June in 2013, 8 June in 2014, 4 June in 2015). This was done to accommodate behavioral observations of 8-13 day-old nestlings and corticosterone sampling of 12-day old nestlings, both of which occurred in the afternoons (see below).

Nest Monitoring. Nest boxes were checked at least every other day, and always on days when predator trials occurred. During each check, the degree of nest building was noted, or the number and temperature of eggs or chicks were assessed. Unhatched eggs were collected three days after the first egg had hatched. Dead nestlings were collected upon discovery. On Day 6 following hatching, nestlings were individually marked with nail polish and measured (head-bill, flat wing, and mass). On Day 12, nestlings were measured again and banded with a USGS aluminum band. In addition, a small blood sample was taken from the brachial vein. Following banding, nests were left unchecked until nestlings were at least 18 days old in order to reduce the risk of premature fledging. Nestlings that disappeared from the box after 18 days of age were assumed to have fledged.

Capture and Sampling. Adults were captured between 0700 and 1100 each day in order to minimize expected variation due to diel fluctuations in circulating plasma corticosterone (Breuner et al. 1999). During mid-incubation (females only) and the early nestling period (females and males), adults were captured on the nest, either by hand or by the use of an external

observer-controlled trap. A blood sample was drawn by brachial venipuncture within three minutes of capture to assess baseline levels of plasma corticosterone (Wingfield et al. 1982), and again after thirty minutes, by which point stress-induced plasma corticosterone was expected to have reached a peak. During the intervening half-hour, each bird was banded and measured (head-bill, to the nearest 0.01 mm; flat wing, to the nearest 0.5 mm; mass, to the nearest 0.25 g). After the second blood sample was drawn, the adult was released and usually returned to its nest within a matter of minutes. In 2014 and 2015, 12-day old nestlings were similarly sampled at 0 and 30 minutes post-disturbance, but their sampling window was restricted to 1500-1900 each day. In 2013, a single blood sample was drawn from each nestling for genotyping, but no plasma samples were obtained.

Behavioral Observations. To assess whether chronic exposure to predators influenced parental feeding behavior, we measured nest visitation rates during a 30-minute period on a single day when nestlings were between 8 and 13 days old (2013-2014 only). Behavioral observations were performed between 1200 and 1500 each day, and consisted of tallying the number of times that either adult entered the box. During each trial, an observer sat in an open field or a parked vehicle at least 50 meters away from the focal nest box. Pairs were assumed to be comfortable with observer presence when they began entering the nest box without hesitation, and first entry by either adult was used as the start of each trial. In order to reduce the effect of variation due to weather conditions and food availability, behavioral observations were only performed when temperatures were above 18°C and there was no precipitation.

Paternity Analysis. DNA was extracted from red blood cells stored in lysis buffer, or from frozen eggs and nestlings, using the QIAGEN® DNeasy Blood & Tissue Kit. We used multiplex polymerase chain reaction (PCR) to amplify nine microsatellite loci previously developed to assess parentage in this species (Makarewich et al. 2009). Each forward primer was labeled with one of four fluorescent dyes. The nine primer pairs were then distributed among two

master mixes, each of which was designed to accommodate the simultaneous amplification of loci of differing size or dye color. PCR conditions were as follows: an initial denaturation step of 2 min at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 60 s of annealing at either 56°C or 58°C, and 60 s of extension at 72°C. A final elongation step was thereafter performed for 30 min at 72°C. PCR products were analyzed on an ABI 3730 x1 capillary sequencer, and Geneious (v.9.0.5; Kearse et al. 2012) was used to assign alleles.

We used CERVUS (v. 3.0; Kalinowski et al. 2007) to assign genetic sires to nestlings. In every case, the social female was assumed to be the mother of all nestlings in her nest. Single locus mismatches were treated as genotyping errors, while mismatches that occurred at more than one locus were assumed to represent instances of sample misidentification. A male was identified as the genetic father of a nestling if he met one of the following two criteria: (1) CERVUS designated him as the genetic father with greater than 95% confidence; or (2) CERVUS did not assign paternity to any sampled male with 95% confidence, but the male in question was the social father and mismatched the offspring at fewer than two loci (i.e. his genotype was consistent with his being the genetic father). Because sampling of males at our study site was incomplete (e.g. due to nest failure, floaters, etc.), we allowed males captured in one year of study to be candidate sires for nestlings born in any of our study years.

We also used CERVUS to test for deviations from Hardy-Weinberg Equilibrium (HWE) and the presence of null alleles. Loci that did not conform to HWE expectations were removed from subsequent analyses.

Hormone Analysis. Upon collection, blood samples were stored on ice for up to 4-6 hours. Thereafter, we separated red blood cells from plasma by centrifugation. Plasma samples were frozen at -20°C until analysis. Circulating baseline and stress-induced plasma corticosterone were assessed using a miniaturized version of the MP Biomedicals radioimmunoassay (as described in Palacios et al. 2007). All corticosterone concentrations were ln-transformed prior to statistical analysis.

Statistical Analyses. We used linear mixed effects models (*glmer* and *lmer* functions in R (v.3.15)) to test the effects of predator exposure on fitness outcomes of nestlings of differing paternity status. Survival was coded as a binary response, while morphometric data (head-bill, wing length, and mass) and ln-transformed corticosterone concentrations were continuous. We included extra-pair status (EPO or WPO), predator treatment (predator or non-predator), and their two-way interaction as fixed effects. Our predator manipulation resulted in equal site-wide disturbance between the two treatment groups, but generated random variation in disturbance at the level of individual nest boxes. Therefore, in addition to testing for a site-wide effect of predator exposure, we also examined disturbance (defined as the number of times a mount was placed in front of a particular nest box) and predator disturbance (defined as the number of times a predator mount was placed in front of a particular nest box) as potential fixed effects. Finally, we included side of site as a fixed effect to account for any spatial variation in nest success or investment at our study site. Predator treatment, disturbance, predator disturbance, and side of site were never included together in a single model. All other combinations of fixed effects were tested. Nest was coded as a random effect in all models.

Because fitness variation between predator-exposed EPO and WPO might result from differential selection of social or extra-pair sires on predator-exposed sites, we tested whether three characteristics of social and extra-pair sires varied across our treatment groups. We used general linear models (*glm* function in R) to test the effects of predator exposure on male age, size, and stress physiology. Because our predator and non-predator treatments necessarily coincided with spatial variation at our study site, we also considered the side of our site on which each male was nesting as a fixed effect. We examined all social males, all extra-pair males, and pairwise differences between social and extra-pair males in each trait. For nests that had more than one identified extra-pair sire, we averaged trait values together prior to analysis such that each nest had a set of trait values for its social male, and a single composite set of traits for its extra-pair males. We similarly used general linear models to test the effects of predator treatment and side of site on age, size, and stress physiology of females.

Finally, we investigated whether nest visitation rate varied with predator exposure. This, too, could account for apparent fitness differences between EPO and WPO, if parental behavior shifts in a way that favors one type of nestling over the other. We used number of trips to the nest in a 30-minute period as a response variable, and examined several general linear models that incorporated predator treatment, side of site, brood size, and nestling age as fixed effects.

We used Akaike's Information Criterion (AIC; Akaike 1973) to compare models, and treated models with $\Delta\text{AIC} \leq 2$ as equally well-supported. When several models were equally well-supported, we considered the most parsimonious to be the best fit.

Results

Paternity Analysis. Four hundred and forty-nine eggs, comprising 87 nesting attempts, were laid in our study area between 2013 and 2015. Of these, we successfully genotyped 323 offspring from 65 nests. Of the remaining 126 eggs that were not genotyped, thirteen were infertile eggs, and the remaining 113 eggs or nestlings disappeared or failed before the offspring and both parents could be sampled. Fifty of 323 genotyped offspring came from buffer nests not assigned to either treatment group, and were thus excluded from analysis. The remaining 273 genotyped offspring were included in our analyses of survival to fledging. Only those that successfully fledged ($n = 211$) were included in our analyses of growth and stress reactivity.

Overall, fifty-one nests (78%) contained at least one EPO. One hundred fifty-seven offspring (49%) were sired by extra-pair males, of which we were able to identify the sire for one hundred thirty (83%). Rates of extra-pair paternity were similar across both treatments (predator: 69% of nests, 47% of nestlings; non-predator: 79% of nests, 45% of nestlings) and sides of our site (near: 83% of nests, 47% of nestlings, far: 69% of nests, 45% of nestlings). All nine loci used in parentage analysis were in HWE (Table 1.1). Mean expected heterozygosity for these nine loci was 0.7960. Second-parent exclusion probability was 0.9999.

Table 1.1: Summary of nine loci used to assess parentage in Tree Swallows.

Locus	k	N	H _{obs}	H _{exp}	HW	F(Null)
TLE19	18	418	0.840	0.849	NS	0.0046
TLE16	15	418	0.830	0.811	NS	-0.0145
TaBi4	20	417	0.880	0.855	NS	-0.0151
TaBi8	12	417	0.849	0.834	NS	-0.0104
TaBi1	14	417	0.734	0.731	NS	-0.0031
TaBi25	17	417	0.624	0.635	NS	0.0122
TaBi34	24	417	0.873	0.867	NS	-0.0047
Tbi104	12	417	0.791	0.800	NS	0.0028
Tal6	11	416	0.786	0.782	NS	-0.0051

Nestling Fitness Outcomes. We compared 14 models representing our hypotheses for how extra-pair status, predator exposure, nest disturbance, and side of site might influence (1) survival to fledging, (2) growth, and (3) corticosterone physiology (see Appendix: Tables S1-10). Survival to fledging was best explained by nest disturbance, with the probability of fledging being negatively impacted by increased disturbance (Table 1.2). In contrast, variation in nestling growth was best explained by an interaction between extra-pair status and predator treatment, such that predator-exposed boxes yielded EPO that were significantly larger than WPO (all ages and metrics; Figure 1.2; Table 1.2). In contrast, non-predator-exposed boxes yielded either (1) EPO and WPO that were of similar size (all Day 6 models and Day 12 head-bill and wing length) or (2) WPO that were larger than EPO (Day 12 mass; Figure 1.2; Table 1.2). Nestling stress physiology was not significantly influenced by any of our explanatory variables (Table 1.2).

Table 1.2: Summary statistics for best-supported models for metrics of nestling fitness. *EP Status* = extra-pair status, *Pred* = predator treatment, *P* = predator exposure, *NP* = non-predator exposure.

Fitness Metric	Significant Terms in Best-supported Model	Significant Pairwise Differences
Survival to Fledging (n = 273)	Disturbance: p = 0.03	
Day 6 Head-Bill (n = 211)	EP Status*Pred: p = 0.003	EPO P > WPO P: p = 0.007
Day 6 Wing Length (n = 211)	EP Status*Pred: p = 0.009	EPO P > WPO P: p = 0.006
Day 6 Mass (n = 211)	EP Status*Pred: p = 0.0003	EPO P > WPO P: p = 0.005 WPO NP > WPO P: p = 0.04
Day 12 Head-Bill (n = 211)	EP Status*Pred: p = 0.0002	EPO P > WPO P: p = 0.004
Day 12 Wing Length (n = 211)	EP Status*Pred: p = 0.0005	EPO P > WPO P: p = 0.01
Day 12 Mass (n = 211)	EP Status*Pred: p = 0.0001	EPO P > WPO P: p = 0.03 WPO NP > EPO NP: p = 0.04
Baseline Corticosterone (n = 144)	None	
Stress-induced Corticosterone (n = 149)	None	
Corticosterone Stress Response (n = 142)	None	

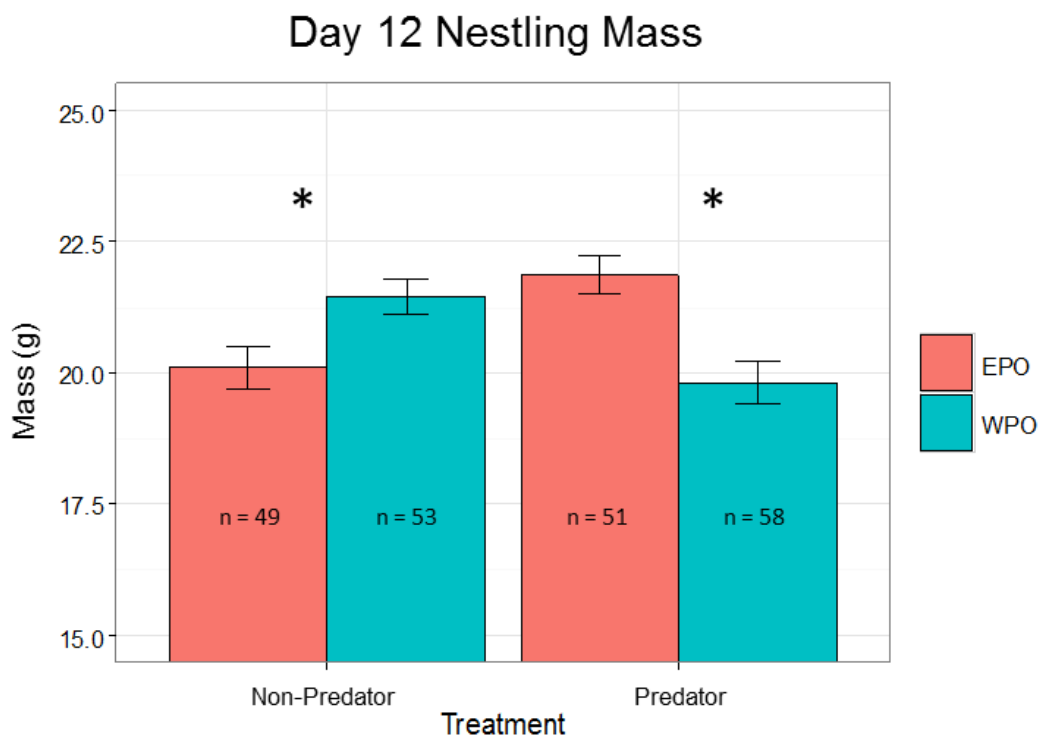


Figure 1.2: Mass (mean \pm SE) on Day 12 post-hatching for EPO and WPO born in non-predator-exposed or predator-exposed boxes. Asterisks indicate significant pairwise differences between EPO and WPO within each treatment group.

Sire Characteristics. We used general linear models to examine how three characteristics (age, size, and stress reactivity) of social and extra-pair males varied with either predator exposure or side of site on which they were nesting. We used predator treatment and side of site as fixed effects, and trait values for social males, extra-pair males, or the pairwise difference in trait value between social and extra-pair males as a response (see Appendix: Tables S11-31). Among social males, age, size, and stress physiology varied with side of site, such that males breeding on the far side of our site were older, larger, and exhibited a higher corticosterone stress response than males living on the near side (Table 1.3). Extra-pair sires breeding on the far side of our site were likewise larger and heavier than extra-pair sires breeding on the near side, and they exhibited a higher corticosterone stress response (Table 1.3). Predator exposure also influenced characteristics of extra-pair sires: extra-pair males at our predator boxes had longer

wings than extra-pair sires from non-predator boxes (Table 1.3). However, pairwise differences between social and extra-pair males were unaffected by either predator treatment or side of site (Table 1.3).

Table 1.3: Summary statistics for best-supported models of male characteristics. *Pred* = predator treatment.

Male Trait	Significant Terms in Best-Supported Model		
	Social Male (n = 55)	Extra-pair Males (n = 31)	Extra-pair Males - Social Male (n = 31)
Age	Side of Site: p = 0.007	None	None
Head-Bill	Side of Site: p = 0.02	Side of Site: p = 0.05	None
Wing Length	Side of Site: p = 0.006	Pred: p = 0.009	None
Mass	Side of Site: p = 0.01	Side of Site: p = 0.01	None
Baseline Corticosterone	None	None	None
Stress-Induced Corticosterone	None	None	None
Corticosterone Stress Response	Side of Site: p = 0.04	Side of Site: p = 0.02	None

Female Characteristics. In contrast to males, female age, size, and stress reactivity did not vary across the two sides of our study site or with predator exposure (see Appendix: Tables S32-42).

Parental Feeding Rates. We used general linear models to examine the effects of predator exposure, side of site, brood size, and nestling age on parental visitation rate. Only nestling age had a significant effect on visitation rate, such that parents visited older nestlings more often (p = 0.02, see Appendix: Table S43).

Discussion

In this study, we investigated whether genetic benefits of extra-pair paternity are context-dependent – that is, whether they are apparent in some environmental contexts, but not others. Specifically, we examined whether experimentally elevated exposure to predators influences the relative fitness of EPO and WPO Tree Swallows. We predicted that EPO would outperform WPO when predation risk was high, and that differences in fitness would be proportionally smaller when predation risk was comparatively low. Indeed, we *did* find that EPO reared under heightened predation risk were larger and heavier than similarly-reared WPO; however, we also found that WPO were heavier than EPO in nests that had not been exposed to predators. This reversal in fitness across environmental contexts falls outside of a genetic benefits framework and is not predicted by *any* existing model that seeks to explain female extra-pair mating in birds. Thus, it demands an explanation.

Genetic benefits models implicitly assume that EPO are of intrinsically superior genetic quality to WPO. If this is the case, then EPO should *always* perform the same as or better than their maternal half-siblings. When this prediction has not been met, (i.e., when WPO have outperformed EPO; as in Hsu et al. 2014), researchers have tended to conclude that extra-pair mating must be non-adaptive in females. Here, we suggest a second possibility, namely that EPO and WPO may *both* be advantageous, but each in a different environment.

Evolutionary biology is replete with examples of alternative phenotypes that vary in fitness across environmental contexts. For example, in the Side-blotched Lizard (*Uta stansburiana*), three color morphs exhibit frequency-dependent selection, such that each color morph performs best when it is comparatively rare (Sinervo and Lively 1996). Likewise, tadpoles of many amphibians are known to develop specialized defensive traits in response to cues that signal heightened predation risk (e.g. Matsunami et al. 2015). While this latter scenario cannot explain fitness differences between EPO and WPO, the size differences that we observed may reflect an underlying genetic difference in the ability to cope with and mitigate stress.

Such a genetic difference could have come about in one of several ways. First, females

might have chosen extra-pair males differently under high and low predation risk. For example, if nestlings possessing specific character traits perform better in a high-predator environment, then females might have biased their choice of extra-pair sires to recruit these characteristics in their offspring (see Branch et al. 2015, Podmokla et al. 2015). Indeed, consistent with this idea, we found that females who bred in predator-exposed boxes chose extra-pair sires possessing longer wings than did females who bred in non-predator-exposed boxes. It is also possible that female mate choice was not strategic at all, but differed between predator and non-predator boxes because of predator-induced changes in encounter rate or opportunity. If, for example, males spend a greater proportion of time away from their next boxes when predation risk is higher (e.g. Tilgar et al. 2011), then females might have greater opportunity to meet and evaluate extra-pair males. If shorter-winged males spend proportionally more time away from the nest site than their longer-winged counterparts, then predator-exposed females may have incidentally encountered and mated with longer-winged males.

It is easy to imagine that long wings might be advantageous to nestlings preparing to fledge into a high-predator environment if they improve flight efficiency or speed. However, because flight performance depends not only on wing length, but on elements of wing shape for which we have no data (e.g. Swaddle and Lockwood 2003), we are unable to evaluate this hypothesis at present. It is also possible that longer wings might be associated with other male traits that were under direct selection by predator-exposed females. One intriguing possibility is that females selected extra-pair males whose offspring might have a greater tendency to disperse away from the natal site. This is especially likely if dispersal propensity is correlated with some aspect of personality that might reasonably modulate extra-pair behavior (e.g. aggression: see Duckworth 2008, boldness: Patrick et al. 2012).

It is interesting to note that we found differences between EPO and WPO in structural size, wing length, and mass. But extra-pair sires in our predator and non-predator treatments only differed in wing length. If the differences in nestling size that we observed resulted solely from genetic variation, then we would have expected to see these fully mirrored in sires. That we did

not suggests a role for non-genetic effects. Female birds have tremendous flexibility in how they equip their offspring to meet the challenges of the world. As the heterogametic sex, females determine whether offspring are male or female. Early-laid eggs often hatch first and early-hatched nestlings often outcompete their siblings (e.g. Anderson 1990). And eggs, unlike placentas, partition each offspring from its mother and its siblings at the time of laying: eggs are discrete packages into which a female can deposit different combinations of nutrients, hormones, and antibodies (e.g. Bentz et al. 2013). The extent to which females have control over these processes is not yet clear, but that they are capable of generating tremendous variation within clutches is apparent. If any one of these processes is coincident with the production of extra-pair young on high-predator sites (e.g. if EPO tend to be male or if they tend to be laid early; e.g. Krist et al. 2005, Vedder et al. 2013), then EPO might have been larger and heavier than WPO as a result. Similar processes could have resulted in heavier WPO on non-predator sites.

Thus, the differences in nestling growth that we observed may have been due to predator-induced variation in both genetic and maternal effects. This scenario would suggest that female Tree Swallows bias investment into the type of nestling that is genetically better disposed to succeed in a given environment.

It is important to note that differences in growth did not translate into improved survival or physiological responsiveness of EPO reared under high predation risk or WPO reared under low predation risk, as might be expected if larger size is correlated with higher fitness. This could be because the stressor that we employed – predation risk – is inherently anticipatory in nature. Certainly, heightened predation risk might be expected to generate stress in adult birds, but for nestlings, whose scope of experience is limited to the nest box, the effects may be less overt. Our nestlings were not challenged in the same way as if they had been exposed to disease or nutritional limitation. And while heightened predation risk might have indirectly induced nutritional stress by altering adult provisioning behavior, it does not appear to have done so in our study. Thus, it seems likely that larger nestlings would have exhibited higher fitness had they been appropriately challenged. Indeed, larger nestlings do exhibit higher post-fledging survival

in our population (this dissertation, Chapter 2).

One final puzzle deserves attention. In comparing characteristics of social and extra-pair males at our study site, we found that males breeding on the far side of our site were older, larger, and exhibited a stronger stress response than males breeding on the near side of our site. In our population, extra-pair sires tended to be males breeding in neighboring boxes (K. K. Hallinger, unpublished data). Thus, the higher quality of extra-pair males at the far side of our site most likely reflects a difference in the pool of available candidate sires rather than any specific bias towards the selection of high-quality extra-pair mates. This idea is supported both by the fact that social male quality showed the same pattern, and that we observed no differences in pairwise characteristics between social and extra-pair males at a single box.

We alternated which side of our site was exposed to predators in successive years of study. Thus, in 2013 and 2015, the far side of our site was exposed to our predator treatment. In 2014, the near side of our site was exposed to predators. Because the near side of our site consistently supported fewer nests than the far side, our aggregate data set included very few predator-exposed nests from the near side of our site. Thus, it is possible that the differences we observed between EPO and WPO on our predator sites were solely a function of their being predominantly sired by high quality males rather than an effect of predator exposure *per se*. If this were the case, then we would also expect to see larger EPO on the far side of our site in 2014, when our non-predator treatment was applied. However, our data from 2014 actually suggest that WPO were larger than EPO under these circumstances, the exact opposite of the pattern we observed in 2013 and 2015 (Figure 1.3). Thus, it appears that we only observed differences between EPO and WPO on the far side of our site, but that the direction of these differences depended on predator treatment.

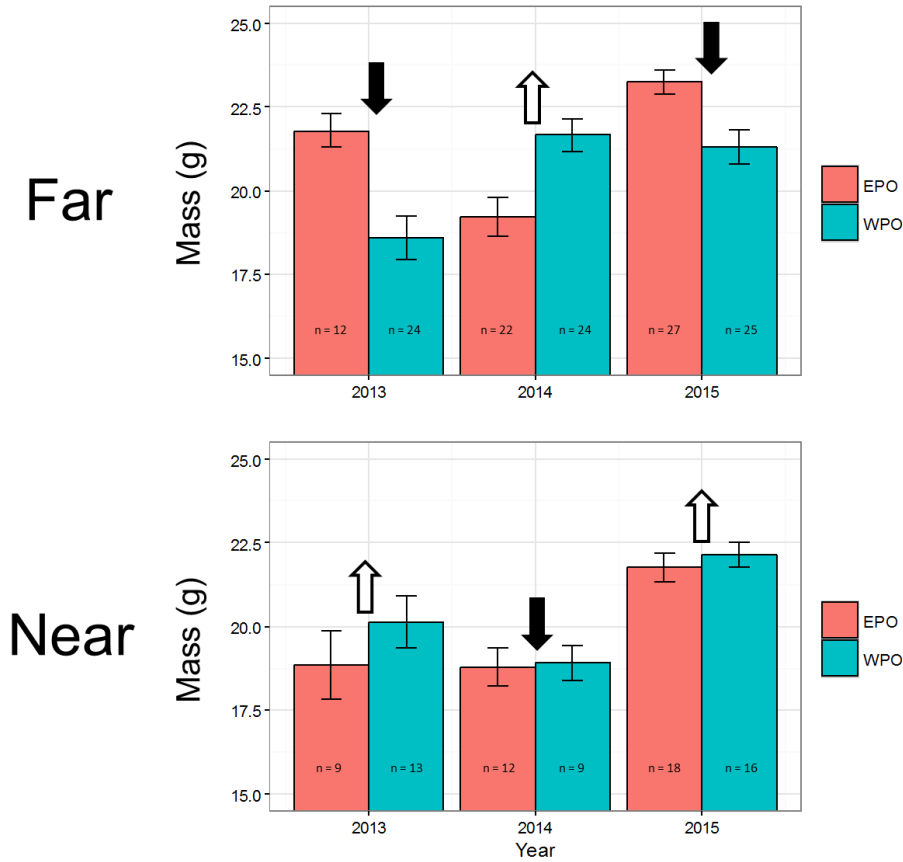


Figure 1.3: Day 12 nestling mass (mean \pm SE) of EPO and WPO born on each side of our study site in 2013-2015. Dark, downward-facing arrows indicate nestlings born in predator-exposed boxes, while white, upward-facing arrows indicate nestlings born in non-predator-exposed boxes. Treatments alternated between the near and far sides of our site in successive years of study.

We are left then with the following scenario: At our site, there exists a spatial gradient of male quality such that larger, older males settle in boxes at one end of our site, while smaller, younger males settle at the other. This difference in quality or environment is perhaps reflected in the lower corticosterone stress response observed in near-side males. When we overlay elevated predator exposure, we observe that EPO are larger than WPO, but only on the side where the quality of available sires is relatively high. In the absence of elevated predator exposure, WPO are larger than EPO. When exposed to predators, females apparently select as extra-pair sires males with longer wings. These longer wings may be under direct selection by females because of advantages in flight performance that they confer to offspring, or they may be

correlated with some other male trait that is preferred or more frequently encountered by females in high-predator conditions. The lack of high-quality males possessing these advantageous traits may explain the universal absence of differences between EPO and WPO on the near side of our site.

One question that remains is why females did not range farther from the nest site in the pursuit of extra-pair sires. If, indeed, offspring quality can be improved by choosing high-quality males from the far end of our site, why then do near-side females not select these males? Past studies have had difficulty identifying extra-pair sires in other Tree Swallow populations, even with intensive sampling of non-territorial males (Dunn et al. 1994, Kempenaers et al. 1999, 2001, Dunn and Whittingham 2007). Thus, it has usually been assumed that extra-pair sires are unsampled floaters, or territory-holders in peripheral populations. Not only did sampled territory holders in our population sire 83% of EPO, but these sires tended to be males from neighboring boxes. In fact, of 81 EPO born in either our predator or non-predator boxes, only 16 (20%) were sired by males breeding in the opposite treatment group.

One possible explanation is that males are more selective in who they mate with than has previously been appreciated (see Bonduriansky 2009, Clutton-Brock 2009, Edward and Chapman 2011, Heinig et al. 2014). Though costs of extra-pair mating have been understudied, it is reasonable to think that disease transmission (Podmokla et al. 2015), sperm depletion (Wedell et al. 2002), and agonistic interactions with cuckolded males (Hoi et al. 2013) might all dissuade males from mating indiscriminately. Additionally, males that devote time to pursuing extra-pair copulations may lose paternity in their own nests (Hill et al. 2011). Thus, it may behoove males to exercise discretion in their choice of female partners. If females on the near side of our site are of similarly low quality as males, then perhaps these females are unable to entice high-quality far-side males into mating. However, we observed no differences in age, size, or stress physiology between females breeding on the two sides of our site.

More likely is that females were restricted in their opportunities to engage in extra-pair encounters or to evaluate extra-pair mates. While mate guarding has generally not been reported

in Tree Swallows (reviewed in Winkler et al. 2011), it has not been explicitly explored in our population. A similar deficit exists in our knowledge of male retaliation for suspected female infidelity. However, either of these male behaviors might dissuade females from pursuing lengthy forays to meet males at great distances from the nest site. Perhaps low quality males more carefully monitor their partners, since they, themselves, may be less likely to secure extra-pair copulations. Indeed, near-side males *did* tend to sire fewer EPO on average than far-side males, despite the fact that overall rates of extra-pair paternity were similar between the two sides of our site (K. K. Hallinger, unpublished data). If the primary avenue of parentage for these males is through within-pair copulations, then these males may guard their females or monitor their activities more closely. This could explain why females mated to low-quality males were unable to mate with high-quality males from more peripheral boxes, in spite of the fact that mating with these males might have allowed them to produce better offspring.

Of equal importance is understanding why females on the far side of our site did not produce an overabundance of the more advantageous type of nestling (EPO when exposed to predators, WPO when exposed to non-predators). One likely explanation is that female physiology is not perfectly adapted to select the most appropriate sire at all times. Although a female may be able to influence the probability that any given male sires her offspring (e.g. by mating with a particular male more frequently or closer to ovulation), at the level of the individual egg, there is likely some degree of chance as to whether successful fertilization occurs and by whom. It is also possible that females are unable to anticipate with precision the environment into which their offspring will fledge. Thus, while EPO may fare better than WPO if predation risk remains high at the natal site, females may seek to diversify their brood in the expectation that conditions may change.

In this study, we have shown that predation risk influences the relative growth of EPO and WPO, and suggest that the ability of females to respond adaptively to environmental challenges depends on the availability of extra-pair sires in possession of specific high-value traits. Our study is the first to report an apparent fitness reversal between EPO and WPO across

environmental contexts, and suggests that each type of nestling may be advantageous in particular circumstances. It will be important for future researchers to better understand the precise mechanisms modulating the variation between EPO and WPO that we observed, as well as the long-term consequences of these differences. Regardless, this study suggests important links between genetic diversity, maternal investment, and environment in patterning extra-pair behavior in birds.

CHAPTER 2

EXTRA-PAIR PATERNITY DOES NOT INFLUENCE LOCAL RECRUITMENT OF FLEDGLING TREE SWALLOWS (*Tachycineta bicolor*)

Abstract

Mating outside of the social pair bond is a common phenomenon in birds. While the benefits to males of extra-pair mating are fairly intuitive, why females should actively participate in such behavior is less straightforward. One of the most frequently investigated hypotheses for active female extra-pair behavior, the genetic benefits hypothesis, proposes that females engage in extra-pair mating in order to improve the genetic quality of their offspring. However, despite hundreds of studies on this topic, no consensus has emerged as to whether and under what circumstances genetic benefits exist. In this study, we used historical banding records and DNA samples to examine whether extra-pair status influences the likelihood of recruitment of Tree Swallows (*Tachycineta bicolor*) into their natal population. We also investigated whether the impact of extra-pair status on recruitment depended on early-life conditions experienced prior to fledging. Overall, we found no evidence to suggest that extra-pair paternity influenced subsequent recruitment, and thus, our results failed to support the genetic benefits hypothesis. Our study is notable in that we (1) examined an important, integrative measure of fitness at an advanced stage of life, (2) simultaneously tested predictions made by several variants of the genetic benefits hypothesis, and (3) allowed for possible environmental modulation of effects. That we observed no patterns in support of the genetic benefits hypothesis suggests that its influence is limited in our population, at least with respect to recruitment. Future research should examine additional fitness endpoints and environmental contexts in which genetic effects might be more profoundly expressed, and should also consider alternative explanations for female extra-pair behavior.

Introduction

Choice of an appropriate mate is one of the most important decisions an individual will make. In socially monogamous species – where breeding occurs in single male-female pairs – mate choice becomes an especially potent selective force because an individual's reproductive success is intimately tied to the quality and character of its mate. Infertility, offspring inviability, loss of parental care, and reduced offspring fitness are all potential costs of non-optimal mate choice (e.g. Slagsvold and Lifjeld 1994, Veen et al. 2001, Morrow et al. 2002). However, individuals may be able to mitigate some of these costs by mating outside of the pair bond.

Extra-pair paternity (EPP) – the siring of offspring by a male other than the social father – is a common phenomenon in birds. Long thought to be paragons of monogamy, the advent of molecular genetics has revealed the presence of EPP in up to 90% of avian species studied (Griffith et al. 2002). The advantage of EPP to cuckolding males is self-evident – more potential partners should offer an opportunity to sire additional offspring – but for females, the benefits are less clear. Yet, females of many species appear willing to engage in extra-pair mating, and may even take an active role in soliciting copulations (e.g. Double and Cockburn 2000). A number of hypotheses have been proposed to explain active female participation in extra-pair mating, but none to date have received broad empirical support. Several of the most widely discussed hypotheses are described below:

(1) Insurance against infertility: Natural rates of infertility are wide ranging across avian species, and may reach as high as 39% in some populations (Morrow et al. 2002). A female paired with an infertile mate might benefit from mating with additional males in order to ensure that some or all of her eggs get fertilized (Griffith 2007). Under this hypothesis, the sole benefit to the female of multiple mating comes from attaining sufficient numbers of sperm to fertilize her eggs. Thus, this hypothesis predicts higher hatchability in clutches sired by greater numbers of males, but predicts no differences in fitness between offspring sired by different individuals.

(2) Genetic benefits: Several studies have investigated the hypothesis that females benefit from extra-pair mating by improving the genetic quality of their offspring. A number of variations on this basic theme have emerged that reflect differences in how genetic benefits may accrue:

(i) Good genes: Under this hypothesis, females engage in extra-pair mating with males of superior genetic quality who will pass on advantageous genes to their offspring (Jennions and Petrie 2000, Neff and Pitcher 2005). This hypothesis predicts that females engage in extra-pair behavior as a way of ‘trading up’ from an inferior social mate, and that extra-pair mates are of higher genetic quality than the males they cuckold. The offspring resulting from extra-pair matings (extra-pair offspring; EPO) are of intrinsically higher genetic quality than their within-pair half-siblings (within-pair offspring; WPO).

(ii) Diverse genes: Females engage in extra-pair mating as part of a strategy to genetically diversify their brood. Under such a ‘bet-hedging’ scenario, a genetically diverse brood may be more likely to have some offspring survive an environmental stressor (e.g. disease) where all members of a more homogenous brood might fail (Yasui 1998, 2001). This hypothesis predicts no difference in fitness between EPO and WPO, but instead that more diverse broods have higher average fitness than their less diverse counterparts.

(iii) Compatible genes: Like the ‘good genes’ hypothesis, the ‘compatible genes’ hypothesis predicts that EPO are of intrinsically superior quality to their maternal half-siblings. However, in contrast, extra-pair males are not expected to be genetically superior to the males they cuckold. Instead, the “best” sire depends on

the genetic composition of the mother (Zeh and Zeh 1996, 1997, Blomqvist et al. 2002). What is best for one female may not be best for another, and genetic compatibility is measured as an optimal degree of (dis)similarity. In addition to predicting a fitness advantage for EPO (relative to WPO in broods of mixed paternity), this hypothesis predicts that females will be more likely to engage in extra-pair mating when paired with a genetically similar mate. More genetically heterozygous offspring should have higher fitness, and heterozygosity should be correlated with paternity status.

(3) Evolutionary constraint: While many of the most prolific hypotheses regarding the existence of EPP have assumed an adaptive benefit to females, some researchers have suggested that extra-pair mating may have a neutral, or even negative, impact on female fitness (Westneat and Stewart 2003, Arnqvist and Rowe 2005). The behavior might instead persist in females due to correlated selection on extra-pair mating in males, or as a result of correlated selection on some other adaptive behavior (e.g. ability to divorce) in females. These ideas have recently been explored in an excellent review by Forstmeier et al. (2014), but only rarely have they been tested empirically (but see e.g. Hsu et al. 2014).

The number of studies evaluating genetic benefits far surpasses those examining either infertility or constraint. Yet, even with hundreds of published studies conducted across dozens of species, no consensus regarding the validity of the genetic benefits hypothesis has emerged. There are several good examples of genetic benefits operating in some populations, but just as many studies fail to report any effect of extra-pair status on offspring fitness (see Akçay and Roughgarden 2007).

The reasons for this discrepancy may be numerous and manifold. First, the effect of extra-pair status on offspring fitness, even if real, is expected to be small, and many studies may simply lack the statistical power to detect it. Second, different studies tend to measure different

‘fitness’ endpoints. Thus, it is possible that genetic benefits exist, but are not equally expressed across all aspects of offspring physiology. A third possibility is that genetic benefits exist, but that their expression is context-dependent (Schmoll 2011). Under this scenario, EPO may have a fitness advantage in some environmental conditions, but not others.

Only a handful of studies have looked for context-dependent genetic benefits of EPP (reviewed in Schmoll 2011), but they have collectively shown promise. For example, Garvin et al. (2006) found that Common Yellowthroat (*Geothlypis trichas*) EPO mounted a stronger cellular immune response than WPO, but only when temperatures during the nestling period were relatively cold. Likewise, Arct et al. (2013) discovered that Blue Tit (*Cyanistes caeruleus*) EPO raised in experimentally enlarged broods mounted a stronger cellular immune response than WPO reared under the same conditions. In control broods left at their natural size, no such difference was found (Arct et al. 2013). Both of these studies (see also Schmoll 2011 for others) lend support to the idea that EPO might out-perform WPO when environmental circumstances prove challenging (but see O’Brien and Dawson 2007).

In this paper, we perform a test of the context-dependent genetic benefits hypothesis in a population of Tree Swallows (*Tachycineta bicolor*) breeding in Ithaca, NY. Using nine years of demographic and genotypic data, we test predictions associated with each variant of the genetic benefits hypothesis for extra-pair behavior (Table 2.1), and then examine whether the relative likelihood of recruitment for EPO vs. WPO varies as a function of early-life environment.

Table 2.1: Predictions made by each variant of the genetic benefits hypothesis (modified from Hsu et al. 2014).

Hypothesis	Offspring Fitness	Nest Fitness	Male Fitness	Explanation
Good Genes	$EPO = WPO_m > WPO_p$	$EPP_m = \text{no } EPP > EPP_p$	Social < Extra-pair	Females mate with superior extra-pair males to acquire better genes for offspring
Diverse Genes	$EPO = WPO_m = WPO_p$	$EPP_m = \text{no } EPP < EPP_p$	Social = Extra-pair	Females mate multiply to acquire diverse genetic combinations of offspring
Compatible Genes	$EPO = WPO_m > WPO_p$	$EPP_m = \text{no } EPP > EPP_p$	Social = Extra-pair	Females mate with dissimilar extra-pair males to produce more heterozygous offspring

EPO = extra-pair offspring; WPO_m = within-pair offspring from singly sired (monogamous; 100% WPO) broods; WPO_p = within-pair offspring from multiply-sired (polyandrous; some EPO) broods; EPP_m = singly-sired extra-pair broods (monogamous; 100% EPO), EPP_p = broods containing a mix of WPO and EPO (polyandrous)

Methods

Study Species. Tree Swallows are insectivorous, migratory songbirds that breed throughout much of North America (Winkler et al. 2011). As secondary cavity nesters, they readily accept artificial nest boxes for breeding, and can be recruited into study populations in large numbers. Their tractability and resistance to disturbance have made them a model organism for addressing a wide range of evolutionary and ecological questions, including those related to EPP (Jones 2003). Tree Swallows have one of the highest EPP rates ever reported in a songbird (up to 89% of nests and 50% of nestlings; Winkler et al. 2011), and as is the case in the wider literature, the reasons for this are unclear. They are especially good candidates for examining the potential role of environment in mediating genetic benefits because their food supply is tightly linked to prevailing weather conditions on the breeding ground (McCarty and Winkler 1999). Previous work has reported high adult and nestling mortality resulting from unseasonably cold temperatures that limit the abundance of flying insects (Hess et al. 2008, Winkler et al. 2013). However, the extent to which developmental temperatures influence recruitment into the breeding population in subsequent years is unknown. Overall rates of recruitment in this species

range from 0.8-12.0% of nestlings, but it is thought that up to 25% of nestlings may survive their first year (Winkler et al. 2011), with the discrepancy between recruitment and survival due to many surviving nestlings settling outside of their natal site (Winkler et al. 2005).

Study Sites. This study took advantage of archival blood samples and historical nesting data collected from a population of Tree Swallows breeding at five sites located in Tompkins County, New York, USA. At each site, between 22 and 260 nest boxes were erected and placed approximately 20 meters apart in suitable habitat for swallow breeding. Though initial sites were established as early as 1985, we restricted our analyses to the years 2002-2010, by which time blood sampling had become standard, and all sites had been established.

Field Methods. In each year of study, nest boxes were monitored beginning in early to mid-April, after swallows had returned to the breeding grounds, but before nest building had begun. Boxes were checked thereafter at variable intervals depending on year, but usually at least once every 2-3 days. During each check, stage of nest building, or the presence and number of eggs or chicks, was recorded. Adults were captured in mist nets prior to egg laying, or in their boxes during incubation or the nestling period. Upon capture, each individual was banded with an aluminum USGS band, and its age and sex were noted when possible. Standardized measures of head-bill length, flat wing length, and mass were made, and a small blood sample was drawn by brachial venipuncture and stored in lysis buffer. Nestlings were similarly banded, measured, and blood sampled between days 7 and 14 post-hatch. Following fledging (which typically occurred between 18 and 22 days post-hatch), nests were checked for the presence and identity of dead nestlings. Additional experiments involving adults and nestlings were carried out by individual researchers throughout the course of our study. Several such nests were excluded from our study, depending on severity of the experimental protocol, and criteria for exclusion are described below (see *Sample Selection*).

Sample Selection. Several variants of the genetic benefits hypothesis predict differences in fitness between EPO and WPO within the same nest (see Table 2.1). In order to test predictions made by these hypotheses, we first used banding records to identify nests that had gone on to produce recruits. From this list, we then excluded any nest for which: (1) the social male had not been identified; (2) blood samples were missing for at least one member of the family (excluding unhatched eggs or nestlings who had died prior to fledging); or (3) the anticipated fitness consequences of an experimental treatment were deemed to be significant (e.g. feather clipping, brood manipulation, etc.). After applying such filters, we were left with a total of 53 candidate nests that could be used to test the influence of extra-pair status on recruitment probability (hereafter, ‘recruit nests’).

Because some variants of the genetic benefits hypothesis (notably, the ‘diverse genes’ hypothesis) make no predictions regarding the relative fitness of half-siblings, but instead predict fitness consequences of EPP at the level of the nest, we also sought to identify a representative group of nests that had experienced the same average conditions as our recruit nests, but from which no recruits had come. These ‘non-recruit nests’ were identified by applying the same filters as above, but this time to a pool of nests that had not produced recruits (but which had fledged at least one offspring). From this pool, we attempted insofar as was possible to match non-recruit nests with recruit nests according to lay date, year, and site. This left us with two groups of nests of similar composition that could be compared to look for differences in extra-pair behavior between nests that had produced a successful recruit and those that had not.

One important goal of this study was to determine whether the relative probability of recruitment for EPO vs. WPO depends on conditions experienced during development. Testing this hypothesis requires that we have a good baseline understanding of how developmental environment influences recruitment more generally. But both groups of nests detailed above represent a (potentially) non-random subset of Tree Swallow nests in our population. If recruit nests differ from non-recruit nests more broadly (e.g. if recruits tend to be produced most often from early season nests), then our sampling protocol will effectively mask important

determinants of recruitment. We therefore examined recruitment in a random subset of nests (hereafter, ‘control nests’) that had been treated in a standardized way over the course of our study period (2002-2010). These nests were used as controls for a larger study of latitudinal life history variation across the *Tachycineta* genus, and were guarded from disruptive manipulations. Examining recruitment in these nests enabled us to identify factors that contribute to successful recruitment in our population, and to assess the expected probability of recruitment for each of our focal nestlings.

Genetic Analysis. DNA was extracted from red blood cells stored in lysis buffer using the QIAGEN® DNeasy Blood & Tissue Kit. For each sample, we used polymerase chain reaction (PCR) to amplify nine highly variable microsatellite loci developed to assess parentage in this species (Makarewich et al. 2009). Forward primers were labeled using one of four fluorescent dyes, and PCR multiplexes were designed to accommodate simultaneous amplification of loci of differing size range and dye. A total of two multiplex reactions were run for each sample, one containing five primer pairs, the other containing four. Each multiplex reaction consisted of 1 µl PCR buffer, 1.30 µl MgCl₂, 0.12-0.36 µl of each primer pair, 0.20 µl dNTPs, 0.10 µl Taq polymerase, and 1 µl DNA. Nuclease-free water was used to bring the final volume of each reaction to 10 µl. PCR conditions were as follows: an initial denaturation step of 2 min at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 60 s of annealing at either 56°C or 58°C, and 60 s of extension at 72°C. A final elongation step was thereafter performed for 30 min at 72°C. PCR products were analyzed on an ABI 3730 x1 capillary sequencer, and Geneious (v.9.0.5; Kearse et al. 2012) was used to assign alleles.

We determined nestling sex using a P2/P8 sexing protocol with a *HaeIII* digest (after Whittingham and Dunn 2000). Briefly, we performed PCR using 1 µl 10x PCR buffer, 0.60 µl MgCl₂, 1.3 µl of each primer, 0.2 µl dNTPs, 0.10 µl Taq polymerase, and 1 µl DNA. Reactions were brought to a final volume of 10 µl using nuclease-free water. PCR conditions were as follows: an initial denaturation step of 1 min at 94°C, followed by 34 cycles of 30 s of

denaturation at 94°C, 45 s of annealing at 46°C, and 45 s of extension at 72°C, and finally, 2 cycles of 30 s at 94°C, 45 s at 47°C, and 5 min at 72°C. Z- and W-specific alleles of the CHD1 gene amplified by the P2/P8 primers are of similar size in this species, and are thus difficult to resolve using gel electrophoresis. We overcame this problem by digesting PCR products with *HaeIII* (Griffiths et al. 1996), thereby shortening the Z-specific PCR product and enabling full resolution of sex-specific alleles. Each digest consisted of 1 µl nuclease-free water, 1 µl 10x PCR buffer, 1 µl of restriction enzyme, and 7 µl PCR product. Digests were incubated for 3 hours at 37°C, followed by an inactivation step of 20 min at 80°C, after which PCR products were visualized using gel electrophoresis.

Paternity Analysis. We used CERVUS (v. 3.0) to assign genetic fathers to our focal nestlings. Because we genotyped only those males who were associated with our focal nests, our ability to identify extra-pair sires was limited. Nonetheless, we were able to assess the likelihood of each social male being the sire of the nestlings in his nest, and thereby distinguish EPO from WPO.

Each social mother was assumed to be the genetic mother of all offspring in her nest, as egg dumping is thought to be rare in this species (Winkler et al. 2011). Single locus mismatches were treated as genotyping errors; mismatches occurring at more than one locus were assumed to represent instances of sample misidentification. Each social father was assigned genetic parentage of a nestling in his nest if one of the following sets of conditions was met: (1) CERVUS designated him as the genetic father with greater than 95% confidence; or (2) CERVUS did not identify any candidate father with 95% confidence, but the social father mismatched his putative offspring at fewer than two loci (i.e. his genotype was consistent with his being the genetic father).

We also used CERVUS to test for deviations from Hardy-Weinberg Equilibrium (HWE) and the presence of null alleles. Loci that did not conform to HWE expectations were removed from subsequent analyses.

Statistical Analyses. In order to discover how early-life experience might influence subsequent probability of recruitment, we constructed Generalized Linear Mixed Models (GLMMs; *glmer* function in R (v.3.15)) designed to examine the relationship between a number of life history, environmental, and individual attributes and recruitment of nestlings. We used our control nest (see *Sample Selection*) data set, and included only those nestlings known to have successfully fledged. Successful recruitment was coded as a binary response, and nest was included as a random effect in all models. Fixed effects included in the full (most parameterized) model are listed, along with their rationale for inclusion, in Table 2.2. After running this model, we then used the *drop1* function in R to perform stepwise backwards removal of fixed effects in order to select the best supported model for use in subsequent analyses. We used Akaike's Information Criterion (AIC; Akaike 1973) to compare models, and treated models with $\Delta\text{AIC} \leq 2$ as equally well-supported. Whenever removal of a fixed effect resulted in two models of equivalent support, we chose the more parsimonious model. Stepwise removal of fixed effects continued until removing additional terms resulted in an increase in AIC score of at least 2.

Table 2.2: List of fixed explanatory variables included in most parameterized model exploring the relationship between early-life experience and recruitment in Tree Swallows.

Fixed Effect	Definition	Possible Values	Predicted Effect on Recruitment
Julian Lay	Date of clutch initiation relative to Jan 1	≥ 1 (Jan 1 = 1)	Increased probability of recruitment from earlier nests
Maternal Age	Whether mother was one year old (SY) or older (ASY)	SY, ASY	Decreased probability of recruitment from nests reared by 1 year-old females
Maternal Origin	Whether mother was banded at one of our sites as a nestling or an adult	local, foreign	Increased probability of recruitment from nests reared by local females
Clutch	Number of eggs in clutch	≥ 1	Increased probability of recruitment from nests producing more eggs if productivity signals female quality, decreased probability of recruitment if per capita resources are reduced
Brood	Number of eggs hatched	≥ 1	Increased probability of recruitment from nests producing more nestlings if productivity signals female quality, decreased probability of recruitment if per capita resources are reduced
Ave Max Temp (Incubation)	Average daily maximum temperature during incubation ^{1,2,3}	any number	Increased probability of recruitment from nests incubated under warmer average temperatures
Ave Max Temp Swing (Incubation)	Average (absolute) change in daily maximum temperature during incubation ^{1,2,3}	≥ 0	Increased probability of recruitment from nests incubated under less variable temperatures
Ave Max Temp (Early Nestling)	Average daily maximum temperature during nestling days 0-8 ^{1,3}	any number	Increased probability of recruitment from nests reared under warmer average temperatures
Ave Max Temp Swing (Early Nestling)	Average (absolute) change in daily maximum temperature during nestling days 0-8 ^{1,3}	≥ 0	Increased probability of recruitment from nests incubated under less variable temperatures
Ave Max Temp (Late Nestling)	Average daily maximum temperature during nestling days 9-17 ^{1,3}	any number	Increased probability of recruitment from nests reared under warmer average temperatures

Table 2.2 (Continued)

Nestling Wing Length	Estimated or measured flat wing length on day 9 post-hatching ⁴	≥9 mm	Increased probability of recruitment by longer-winged nestlings
Nestling Mass	Estimated or measured mass on day 9 post-hatching ⁴	≥3.5 g	Increased probability of recruitment by heavier nestlings

¹ Historical weather data were obtained from the Game Farm Road Weather Station in Ithaca, NY (<http://www.nrcc.cornell.edu/wxstation/ithaca/ithaca.html>).

² Incubation was defined as a period lasting from the date of clutch completion through the day prior to estimated hatch.

³ Because most nests were not checked frequently enough to know the precise date of hatching, we estimated hatch date according to the following rules: nest checks bracketing hatching established the potential range of days on which hatching could have occurred. To parse among the remaining possibilities, we assumed an average incubation period of 13 days post-clutch completion. If this date fell within our known range of possibilities, it was assigned as nestling day 0. If our estimated hatch date fell outside of the known range of possibilities, then the closest possible hatch date was selected. For example, if eggs were present on 15 May and nestlings were present on 18 May, we assumed that hatching could have occurred on either 16, 17, or 18 May. If adding 13 days to the clutch completion date resulted in an estimated hatch date of 17 May, then 17 May was assumed to be the hatch date. If adding 13 days to the clutch completion date resulted in an estimated hatch date of 14 May, then 16 May was assumed to be the hatch date.

⁴ Over the course of our study period, typical age of nestling measurement fell between 6 and 14 days of age, but varied between years. In order to allow for easy comparison of growth metrics across all nestlings, we standardized all nestling measurements to Day 9 post-hatch. To do this, we used control nests from 2009, for which there was a particularly good sequence of repeated measurements of individuals at Days 3, 6, 9, and 12. We assumed that growth was fairly linear over these three-day intervals, and therefore calculated linear growth constants for Days 6-9 and Days 9-12 by regressing flat wing length or mass against age for each individual over each interval. We averaged these individual slope values to arrive at the following growth constants: flat wing length: Days 6-9: 5.6 mm per day, Days 9-12: 5.97 mm per day; mass: Days 6-9: 2.04 g per day, Days 9-12: 0.62 g per day. We then added or subtracted the appropriate number of days' worth of growth for each of our control nestlings to arrive at expected Day 9 measurements. Most nestlings were measured between Days 6 and 12, but a few were measured on Nestling Day 13 or 14. We treated these similarly to those measured between Days 9 and 12. Minimum values represent the smallest measurements for Day 9 nestlings observed in our data.

In order to investigate potential interactions between EPP and early-life conditions on recruitment, we fitted our most parsimonious model of recruitment to our focal recruit and non-recruit nests using the *predict* function in R. This allowed us to generate an expected recruitment probability for each focal nestling that took into account the specific predictor variables identified as having the greatest effect on recruitment. While our data did not allow us to test predictions related to the relative fitness of social vs. extra-pair males (due to sparse sampling of non-focal males in our population), we were able to test predictions concerning individual- and

nest-level fitness for each variant of the genetic benefits hypothesis (see Table 2.1, Fig. 2.1).

For good genes and compatible genes models, which predict differences in fitness between nestlings of differing extra-pair status, we constructed GLMMs that used recruitment as a binary response variable, and expected recruitment probability, individual extra-pair status, and their two-way interaction as fixed effects. We contrasted models in which WPO from singly-sired broods (WPO_m) were coded as being equivalent to WPO from mixed-paternity broods (WPO_p) with models in which WPO_m were assumed to be equivalent to EPO. We did this in order to test for both a simple difference between EPO and WPO, and to allow for the possibility that females mated to high-quality males may not have benefitted from engaging in extra-pair behavior. We ran models that included all nestlings fledged from recruit nests, and also divided our data set into male and female subsets. Nest was coded as a random effect in all models.

For diverse genes models, which predict differences in fitness between nests sired by different numbers of males, we used General Linear Models (GLMs) to examine recruitment at the level of the nest. Realized recruitment again served as a binary response variable, but in this instance, reflected whether at least one fledgling from a nest had returned to its natal site. The expected probability that at least one fledgling from a given nest would return was calculated using individual expected recruitment probabilities according to the following formula:

$$P(\text{at least one fledgling recruits}) = 1 - P(\text{zero fledglings recruit})$$

$$P(\text{zero fledglings recruit}) = (1 - P(\text{Fledgling 1 recruits})) * (1 - P(\text{Fledgling 2 recruits})) * \dots \\ \dots(1 - P(\text{Fledgling } i \text{ recruits}))$$

where i corresponds to the number of fledglings from a given nest

Because diverse genes models posit that brood diversity, rather than extra-pair paternity *per se*, improves fitness, we did not expect nestlings of extra-pair status to be more likely to

recruit. Instead, we predicted that nests of mixed paternity (sired by more than one male) would be more likely to have at least one fledgling return. For nests sired entirely by the social father (all WPO) and nests containing both WPO and EPO, assignment of nest paternity was straightforward. For nests sired entirely by (unidentified) extra-pair males, we visually inspected offspring genotypes to determine whether more than one male had contributed to the brood. Nests for which at least three paternal alleles were present at a single locus across all members of the brood were coded as being of mixed paternity. Nests for which fewer than three paternal alleles were present at each locus were coded as having been singly sired. For our diverse genes GLMs, we examined independent effects of expected recruitment probability and nest paternity, as well as the two-way interaction of these terms. We again ran three candidate model sets, one that included all genotyped nestlings, one that included only female nestlings, and one that included only males. For sex-specific models, nest paternity and recruitment probability were determined independently for each sex. Thus, a multiply sired brood could contain multiply sired females and singly sired males (or any equivalent permutation). For all models, we used Akaike's Information Criterion (AIC) to rank models, and treated models with $\Delta AIC \leq 2$ as equally well-supported.

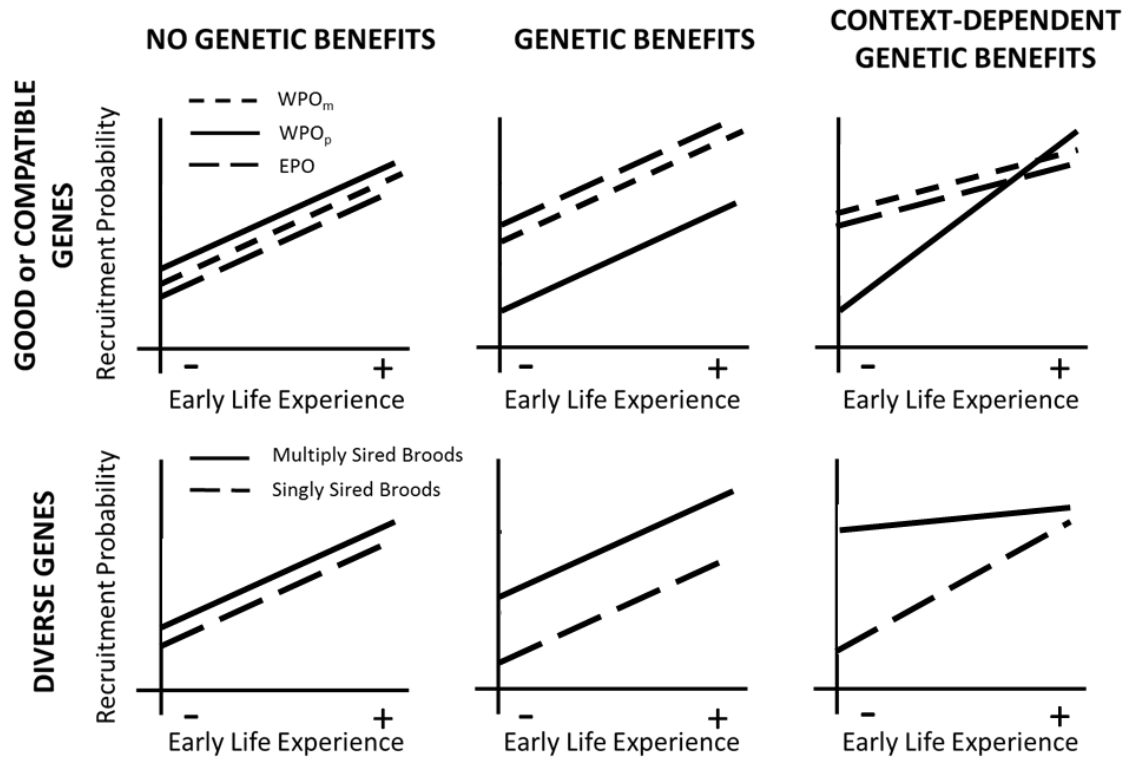


Figure 2.1: Schematic representation of alternative hypotheses evaluated in the present study. Early-life experience is a gradient of environmental and individual attributes that either favor (+) recruitment or discourage (-) recruitment. EPO = extra-pair offspring; WPO_m = within-pair offspring from singly sired (monogamous; 100% WPO) broods; WPO_p = within-pair offspring from multiply-sired (polyandrous; some EPO) broods.

Results

Factors Predicting Recruitment. We examined patterns of recruitment in 596 nestlings fledged from 129 control nests over a period of nine years. Of the 13 early-life variables we identified as having the potential to influence subsequent recruitment of nestling Tree Swallows, our best supported model retained four: Julian lay date, clutch size, average temperature swing during incubation, and nestling mass were significant predictors of recruitment. Specifically, nestlings had a higher probability of recruiting when (1) they fledged from a nest initiated earlier in the breeding season, (2) they fledged from a nest containing fewer eggs, (3) their nest encountered greater ambient temperature variability during incubation, and (4) they were of greater mass (Table 2.3).

Table 2.3: GLMM results for best-supported model exploring the effects of early-life environment on recruitment probability of nestling Tree Swallows. Individual variables listed in Table 2 were eliminated using stepwise backwards removal until most parsimonious model AIC (=452.29) was achieved. Nest was included as a random effect in all models. $n = 596$ fledglings segregated among 129 nests over a period of nine years (2002-2010).

Variable	Estimate	SE	P	Significance
Intercept	0.43	1.05	0.68	
Julian Lay	-0.37	0.19	0.06	
Clutch	-0.44	0.18	0.02	*
Ave Max Temperature Swing (Incubation)	0.39	0.15	0.009	*
Nestling Mass	0.40	0.15	0.008	*

Paternity Analysis. In total, we genotyped 50 recruits and 125 non-recruits from 40 recruit nests and 137 non-recruits from 31 non-recruit nests for use in our final analysis. One-hundred forty-four additional genotyped nestlings were excluded from analysis. Of these, three mismatched their putative mother at more than one locus. Sixty-one died prior to fledging or were not definitively known to have fledged. Their genotypes were used to assess nest-level patterns of parentage, but were otherwise excluded from analysis. Sixty nestlings were excluded owing to the unavailability of mass data, and consequently, an inability to generate an expected recruitment probability. Twenty fledglings came from nests that had been significantly disturbed by researchers. Although siblings from excluded nests were included in good/compatible genes models when possible, nests containing excluded nestlings were removed from diverse genes models.

Of the remaining 312 genotyped fledglings, 156 were extra-pair and 156 were within-pair. Sixty out of our 71 nests contained at least one EPO. Nine contained only EPO. Of the nine microsatellite loci used to genotype individuals, one (TaBi8) deviated from HWE expectations and was thus removed from our data set. Use of the remaining eight loci (Table 2.4) resulted in a mean expected heterozygosity of 0.7915 and a combined second-parent exclusion probability of 0.9998.

Table 2.4: Summary of eight loci used to assess parentage in Tree Swallows.

Locus	k	N	H _{obs}	H _{exp}	HW	F(Null)
TLE16	13	632	0.797	0.794	NS	-0.0014
TLE19	17	632	0.850	0.864	NS	+0.0083
TaBi1	12	632	0.774	0.762	NS	-0.0101
TaBi4	22	632	0.859	0.862	NS	+0.0014
TaBi25	17	632	0.680	0.674	NS	-0.0074
TaBi34	26	632	0.891	0.899	NS	+0.0044
Tbi104	12	632	0.710	0.708	NS	+0.0004
Tal6	11	632	0.783	0.769	NS	-0.0105

Genetic Benefits. We fit our best-supported recruitment model to genotyped offspring to generate an expected probability of recruitment for each of our focal fledglings. A high expected recruitment probability indicated that a fledgling’s early-life conditions were favorable for recruitment, while a lower expected recruitment probability indicated that early-life conditions were less favorable for recruitment. Individual expected recruitment probabilities ranged from 0.01 to 0.37 (mean \pm SD = 0.13 \pm 0.06). Nest-level expected recruitment probabilities ranged from 0.04 to 0.82 (mean \pm SD = 0.44 \pm 0.19). We found no evidence that extra-pair status influenced realized recruitment, either independent of or in interaction with early-life conditions (Table 2.5a). Nor did we find any evidence that nests of mixed paternity were more likely to produce a recruit (Table 2.5b). This was true when all nestlings were considered together (Table 2.5) and when we considered males and females separately (Table 2.6).

Table 2.5: (a) Model selection results for good/compatible genes GLMMs; (b) model selection results for diverse genes GLMs. The symbol ‘+’ refers to an additive effect. The symbol ‘*’ refers to an interactive effect. The best-supported model in each candidate model set is italicized. *EP Status* = extra-pair status; *EPO* = extra-pair offspring; *WPO_m* = within-pair offspring from singly sired (monogamous; 100% WPO) broods; *WPO_p* = within-pair offspring from multiply-sired (polyandrous; some EPO) broods; *k* = number of estimable parameters; *AIC* = Akaike’s Information Criterion, *l* = model likelihood, *w_i* = relative model weight.

(a) Good/compatible genes (N = 175 fledglings)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
Expected Recruitment + EP Status (<i>WPO_m</i> = <i>WPO_p</i>)	3	0	1.00	0.30
Expected Recruitment * EP Status (<i>WPO_m</i> = <i>WPO_p</i>)	4	0.7	0.70	0.21
<i>Expected Recruitment</i>	2	0.9	0.64	0.19
Expected Recruitment + EP Status (<i>WPO_m</i> = EPO)	3	1.3	0.52	0.15
Expected Recruitment * EP Status (<i>WPO_m</i> = EPO)	4	1.3	0.52	0.15

(b) Diverse genes (N = 69 nests)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
<i>Expected Recruitment</i>	2	0	1.00	0.51
Expected Recruitment * Nest Paternity	4	1.1	0.58	0.30
Expected Recruitment + Nest Paternity	3	2.0	0.37	0.19

Table 2.6: (a) Model selection results for sex-specific good/compatible genes GLMMs; (b) model selection results for sex-specific diverse genes GLMs. The symbol ‘+’ refers to an additive effect. The symbol ‘*’ refers to an interactive effect. The best-supported model in each candidate model set is italicized. *EP Status* = extra-pair status; *EPO* = extra-pair offspring; *WPO_m* = within-pair offspring from singly sired (monogamous; 100% WPO) broods; *WPO_p* = within-pair offspring from multiply-sired (polyandrous; some EPO) broods; *k* = number of estimable parameters; *AIC* = Akaike’s Information Criterion, *l* = model likelihood, *w_i* = relative model weight.

(a) Good/compatible genes

Females (N = 94 fledglings)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
<i>Expected Recruitment</i>	2	0	1.00	0.41
Expected Recruitment + EP Status (WPO _m = WPO _p)	3	1.8	0.41	0.17
Expected Recruitment + EP Status (WPO _m = EPO)	3	1.9	0.39	0.16
Expected Recruitment * EP Status (WPO _m = EPO)	4	2.1	0.35	0.14
Expected Recruitment * EP Status (WPO _m = WPO _p)	4	2.6	0.27	0.11

Males (N = 81 fledglings)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
Expected Recruitment + EP Status (WPO _m = WPO _p)	3	0	1.00	0.41
<i>Expected Recruitment</i>	2	1.4	0.50	0.21
Expected Recruitment * EP Status (WPO _m = WPO _p)	4	1.6	0.45	0.19
Expected Recruitment * EP Status (WPO _m = EPO)	4	2.8	0.25	0.10
Expected Recruitment + EP Status (WPO _m = EPO)	3	2.9	0.23	0.09

(b) Diverse genes

Females (N = 65 nests)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
<i>Expected Recruitment</i>	2	0	1.00	0.48
Expected Recruitment + Nest Paternity	3	0.5	0.78	0.38
Expected Recruitment * Nest Paternity	4	2.5	0.29	0.14

Males (N = 62 nests)

Model	<i>k</i>	ΔAIC	<i>l</i>	<i>w_i</i>
<i>Expected Recruitment</i>	2	0	1.00	0.58
Expected Recruitment + Nest Paternity	3	1.3	0.52	0.30
Expected Recruitment * Nest Paternity	4	3.2	0.20	0.12

Discussion

In this paper, we evaluated several variants of the genetic benefits hypothesis for EPP in a long-established population of Tree Swallows. Using nine years of nest records and archival DNA, we investigated the hypotheses that (1) EPO would be more likely to survive and recruit into their natal population than their within-pair half-siblings (good/compatible genes), and (2) that nests sired by multiple males would be more likely to produce a recruit than those sired by a single male (diverse genes). In neither case did we find any support for the idea that EPP significantly influences the likelihood of eventual recruitment.

These results may be unsurprising when considered alongside other studies of EPP in birds. Although the genetic benefits hypothesis offers an attractive explanation for the widespread incidence of active female extra-pair mating, it has only sometimes found empirical support (Akçay and Roughgarden 2007). Thus, our results are in keeping with the majority of studies that have explicitly looked for evidence of genetic benefits. However, the reasons why this should be so require explanation.

One possibility is that genetic benefits exist, but that we were unable to detect them. This could have occurred as a simple result of small sample or effect size. Lack of statistical power is an unsatisfying answer, but it is one that has plagued the field for decades (Griffith et al. 2002, Akçay and Roughgarden 2007). However, an examination of sample sizes reported in the 121 studies compiled by Akçay and Roughgarden (2007) suggests no relationship between sample size and the reporting of genetic benefits, as would be expected if low statistical power provided a general explanation for the phenomenon (genetic benefits observed: mean n averaged across 54 studies = 339 nestlings, 73 nests; no genetic benefits observed: mean n averaged across 52 studies = 447 nestlings, 87 nests). Indeed, our sample size ($n = 312$ nestlings, 71 nests) is very much in line with that of other studies that have reported positive results.

It is also possible that some aspects of fitness are more readily influenced by extra-pair status or genetic diversity than others. If, for example, genetic benefits of EPP manifest chiefly as effects on immune function, then researchers comparing coloration of EPO and WPO may be

less likely to find an effect. Without knowing precisely why females engage in extra-pair behavior, it is difficult to predict *a priori* which facets of fitness are most likely to be affected. We hoped to bypass this concern by examining a long-term fitness metric that necessarily integrates a large number of physiological and behavioral endpoints. For a relatively short-lived, migratory species like the Tree Swallow, the risk of mortality in the first year of life is substantial (upwards of 75%; Winkler et al. 2011). Recruitment into the natal population requires that an individual learns how to fly and forage independently, completes its first pre-basic molt, undergoes migration along an unfamiliar route of at least 2000 km each way, copes with predators, disease, and inclement weather, and acquires a nest site and mate. Successful navigation of each stage of this annual cycle depends on the proper functioning of a vast number of complex immunological, hormonal, metabolic, and neurological pathways. If any one of them is improved as a function of EPP in a way that makes successful recruitment more likely, then EPO should have recruited into our population at higher rates than WPO. And yet, they did not.

We assumed that recruitment rates would be highly sensitive to small variations in morphology, physiology, and behavior, but it is also possible that this particular endpoint is instead driven by a high degree of chance. Under this scenario, EPO might have been genetically superior to WPO, but not in a way that improves their probability of successful recruitment. Because mortality is so high during this life stage, and the challenges so severe, any small differences in quality between EPO and WPO might have been effectively masked by larger stochastic events, such as encountering an intense storm during migration. If this is the case, then we would expect studies examining recruitment in species with similar life histories to report a preponderance of negative results. However, this does not seem to be the case (Schmoll et al. 2005, Sardell et al. 2011, Hsu et al. 2014, Bowers et al. 2015, but see Gerlach et al. 2012). In fact, in the only previous study that has compared recruitment rates of EPO and WPO in a migratory species (House Wrens: *Troglodytes aedon*), EPO were found to recruit at a higher rate than WPO (Bowers et al. 2015).

In this study, we used recruitment into the natal population as a proxy for survival. Past

research in this population has shown that only a small fraction of surviving fledglings return to their natal site, with many more tending to disperse to nearby areas within 10 km of where they were born (Winkler et al. 2005). Thus, many surviving fledglings likely dispersed outside of our study area. If EPO and WPO are equally likely to disperse, then the lack of a difference in recruitment between EPO and WPO should reflect a lack of difference in survival. However, if EPO are more dispersive than WPO (or vice versa), then the more dispersive type of fledgling may have been underrepresented in our sample of recruits. We cannot at present rule out this possibility, and indeed, recent empirical work in other species suggests that it deserves further attention. For example, in Great Tits (*Parus major*), exploratory behavior has been linked to promiscuity, such that bolder males are more likely to sire EPO (Patrick et al. 2012). If these EPO in turn exhibit greater exploratory behavior than their maternal half-siblings, perhaps they are more likely to disperse or to colonize new environments. In a densely populated or suboptimal area, investment in EPO might be one way to give offspring their best chance of survival and successful recruitment. For females breeding in high quality habitat capable of supporting additional individuals, investment in less dispersive WPO might be more advantageous. Though this idea has never, to our knowledge, been investigated in the context of EPP, it has been offered as an explanation for biased sex ratios in several taxa (e.g. insects: Herre 1987, birds: Komdeur et al. 1997), and represents but one possibility for how extra-pair status might interact with subsequent behavior and fitness (see also Sardell et al. 2011).

Our data clearly show that genetic benefits of EPP are not constitutively present in our population, but it remains an open question whether the reverse is also true. It is possible that genetic benefits are only sometimes apparent, and that their appearance is mediated by environment (Schmoll 2011). We tested this idea by indexing early-life conditions experienced by nestling Tree Swallows, and looking for interactions between EPP and the *a priori* chances of successful recruitment. When we did this, we found that actual recruitment was best explained by early-life conditions alone. Only a handful of studies have attempted to examine interactions between EPP and environment, and most of these have supported the idea of context dependence

(Schmoll 2011). That our study does not may be a reflection of the particular environmental variables that we chose to examine. For example, early spring weather on the breeding grounds may be a more important source of variation in first-year survival than conditions in the nest, but we did not consider environmental variables beyond fledging. Before dismissing the idea of context-dependent genetic benefits in our population, it will be important to examine additional sources of environmental variation as well as additional fitness endpoints.

Alternatively, our results may lend support to the small, but growing, body of work that suggests EPP may exist chiefly as a conduit for male reproduction, with active female participation (where it exists) being a by-product of selection on males (Westneat and Stewart 2003, Arnqvist and Kirkpatrick 2005, Forstmeier et al. 2011). Studies reporting a lack of genetic benefits are consistent with this hypothesis, as are a few studies which have shown a depression in fitness of EPO as compared to their maternal half-siblings (e.g. Hsu et al. 2014). However, it remains unclear why genetic constraint should lead to such vast variation in extra-pair behavior across species. For example, extra-pair rates in Tree Swallows are quite high, not only among birds in general, but even among other members of the same genus (Griffith et al. 2002, Ferretti 2010). Why this should be so demands further scrutiny.

Overall, our study adds to the growing body of literature that has failed to support the genetic benefits hypothesis for EPP in birds. There are certainly explanations for our results that exist within a genetic benefits framework (several of which are outlined above), but at the very least, they underscore the complexity of the issue at hand. We used a long-term, integrative measure of fitness and investigated several variants of the genetic benefits hypothesis. We examined effects of EPP both independent of and in interaction with environmental conditions during nestling development. And in no instance did we detect evidence of genetic benefits in our population. Future researchers would benefit from examining additional environmental variables that might be important mediators of fitness, as well as alternative hypotheses for the prevalence of this important and enigmatic behavior.

CHAPTER 3

DIFFERENCES IN DEVELOPMENTAL PLASTICITY INFLUENCE LIFETIME REPRODUCTIVE SUCCESS OF WITHIN-PAIR AND EXTRA-PAIR TREE SWALLOWS (*Tachycineta bicolor*)

Abstract

Extra-pair paternity (EPP) is exceptionally common and widespread in birds. Why males should mate multiply is well-understood, but for females, the benefits are less clear. And yet, females of many species tolerate and, in some cases, actively solicit copulations from extra-pair males. Thus, a number of hypotheses have been put forward to explain benefits that females might gain from engaging in this behavior. The ‘good genes’ hypothesis suggests that females mate multiply in order to improve the genetic quality of their offspring. This hypothesis predicts that offspring sired by extra-pair males (extra-pair offspring, EPO) should be fitter than their maternal half-siblings (within-pair offspring, WPO), but this prediction has only sometimes been met. One reason for this might be that environmental conditions vary in such a way as to either mask or reveal differences in offspring quality at different times. Another explanation might be that genetic benefits of EPP do not become apparent until an advanced stage of life, long after most studies have ended. In this study, we addressed both of these concerns by examining the life history of female recruits of known paternity in a population of Tree Swallows (*Tachycineta bicolor*) breeding in New York. In addition to following females throughout their adult lives, we tested whether environmental conditions experienced during development or contemporary conditions experienced as breeding adults interacted with extra-pair status to influence life history outcomes. Contrary to our prediction that EPO would outperform WPO in certain contexts, we found that WPO survived longer and fledged more nestlings during their lifetimes than EPO, but only when they had experienced relatively benign (warmer, less variable) conditions as nestlings. When reared under more stressful conditions, the average fitness of WPO and EPO was similarly low. Overall, EPO life history was remarkably stable across

developmental contexts, while WPO life history was quite flexible. We suggest that this difference in developmental plasticity might represent a subtle benefit of EPP, and that EPO might serve as a low-risk, low-payoff option for females when environmental conditions are especially harsh. This hypothesis has the potential to explain many disparate outcomes in the extra-pair literature and thus should become a target of future study.

Introduction

Avian extra-pair paternity (EPP) presents a puzzle to evolutionary biologists. On the one hand, it is exceptionally common, occurring in up to 90% of all socially monogamous species that have been studied (Griffith et al. 2002). On the other hand, in most of these species, mating is assumed to be under female control (e.g. Lifjeld and Robertson 1992, Double and Cockburn 2000). This is significant, because female fecundity is not expected to improve as a result of mating with several males. Thus, a large body of research has centered on understanding other types of benefits that females might acquire from engaging in this behavior.

Some researchers have proposed that females mate multiply in order to guard against potential infertility of a social mate (e.g. Sheldon 1994, Kempenaers et al. 1999, Griffith 2007). Others have suggested that females may acquire direct material benefits (e.g. additional food, protection, or parenting) from extra-pair males (e.g. Gray 1997, Townsend et al. 2010). But by far the most frequently investigated and broadly applicable hypothesis posits that females mate multiply in order to improve the genetic quality of their offspring (reviewed in Akçay and Rougharden 2007). Such indirect benefits, or “good genes”, might arise because females have effectively “traded up” to superior or more compatible sires. If this is the case, then extra-pair offspring (EPO) should have higher fitness than their maternal half-siblings (within-pair offspring: WPO).

This prediction has been tested hundreds of times in dozens of species, and yet it has received support in fewer than 50% of published studies (Akçay and Rougharden 2007). This lack of consensus has caused some researchers to hypothesize that extra-pair behavior does not

confer any advantage to females at all, but is instead a byproduct of correlated selection on other female behaviors or on extra-pair mating in males (Forstmeier et al. 2014, Hsu et al. 2014, 2015). This argument is reasonable where the good genes hypothesis has failed, but it cannot explain those instances where the good genes hypothesis has found support. In order to understand why female extra-pair mating occurs, it is necessary to reconcile these discrepancies.

Environmental mediation may provide a way forward because it predicts exactly the sort of discrepancies that exist in the extra-pair literature. Under this hypothesis, EPO may be genetically superior to WPO, but any resultant differences in fitness only become apparent under particular suites of environmental conditions (Schmoll 2011). For example, EPO may be better than WPO at surviving temperature extremes, but whether fitness differences are observed will depend on temperatures that offspring experience. In cold weather, EPO will outperform WPO, while more mild weather will mask any differences. The key then is not whether differences between EPO and WPO exist, but instead whether they are revealed.

Only a handful of studies have examined this idea, but these few studies have proved promising. For example, Common Yellowthroat (*Geothlypis trichas*) EPO exhibited a stronger T-cell mediated immune response than WPO, but only in the colder of two study years (Garvin et al. 2006). Similarly, Blue Tit (*Cyanistes caeruleus*) EPO mounted a stronger humoral immune response than WPO when reared in experimentally enlarged broods (Arct et al. 2013). In control broods left at their natural size, no such difference was found. In general, these studies have found that EPO outperform WPO when environmental conditions are challenging, suggesting that EPO may be better at managing and mitigating stress (reviewed in Schmoll 2011). However, this is not a universal finding. Indeed, in Tree Swallows (*Tachycineta bicolor*), EPO grew longer wings when environmental conditions were benign (low parasite abundance and earlier hatching relative to maternal half-siblings; O'Brien and Dawson 2007). In House Wrens (*Troglodytes aedon*), it was WPO who outperformed EPO in two of three study years (Forsman et al. 2008).

In aggregate, these studies suggest that ecological factors play an important role in determining relative fitness of offspring of differing paternity status. And yet, even the small

number of studies that have examined context-dependent genetic benefits have garnered substantial variation in outcomes. This is interesting because it suggests that EPO may be superior under some conditions and that WPO may be superior under others. Rather than one type of nestling being of intrinsically higher value than the other, EPO and WPO may instead represent alternative life history strategies. For instance, one type of nestling might prioritize fecundity over survival, while the other prioritizes survival over fecundity. Which type of nestling does best will depend on the exact environmental conditions it encounters.

In order to investigate these kinds of questions, it is necessary first to examine fitness outcomes of EPO and WPO across the lifespan, and then to place them in an ecological context. But very few studies have tracked EPO and WPO beyond fledging, and even fewer have incorporated responses to environmental conditions. Furthermore, as in the wider literature, the results of these long-term studies show marked variation. In Dark-eyed Juncos (*Junco hyemalis*), both male and female EPO recruits had higher lifetime reproductive success than WPO recruits (Gerlach et al. 2012). But in House Sparrows (*Passer domesticus*), WPO outperformed EPO in this same metric (Hsu et al. 2014). In the single long-term study that incorporated ecological effects, Coal Tit (*Peripatus ater*) EPO were more likely than WPO to recruit from late-season broods (Schmoll et al. 2005), but this difference did not carry over into adult performance (Schmoll et al. 2009).

In this study, we investigate whether female Tree Swallow recruits of known paternity status differ in a series of important life history traits: natal dispersal, breeding dispersal, annual reproductive success, offspring quality, survival, and lifetime reproductive success. For each metric, we incorporate both natal and contemporary weather conditions to determine whether fitness outcomes of EPO and WPO vary as a function of environmental stress. Our study is one of only a few to examine long-term consequences of EPP, and is the first to simultaneously measure responses of EPO and WPO to environmental stress across the lifespan.

Methods

Study Species. Tree Swallows are migratory aerial insectivores that breed throughout much of North America (Winkler et al. 2011). They are an excellent organism in which to undertake studies of extra-pair behavior because they exhibit one of the highest rates of EPP of any species yet studied (~90% of nests and ~50% of offspring; Winkler et al. 2011). As in other species, evidence for genetic benefits of EPP is equivocal, with several studies showing modest evidence of good genes (e.g. Whittingham et al. 2006, O'Brien and Dawson 2007, Stapleton et al. 2007) and others showing no support for this hypothesis (e.g. Lifjeld et al. 1993, Kempnaers et al. 1999, Whittingham and Dunn 2001). Tree Swallows may be particularly prone to environmentally-mediated fitness variation between EPO and WPO because their diet is closely tied to ambient temperature (McCarty and Winkler 1999), and periods of unseasonably cold weather can result in mass adult and nestling mortality (e.g. Hess et al. 2008, Winkler et al. 2013). Nestlings face additional metabolic challenges when temperatures are low because they hatch without the ability to thermoregulate (Marsh 1980, McCarty 1995). Temperature stress around the time that this ability develops (~9-12 days old) can likewise result in death (Winkler et al. 2011). And yet, in spite of their sensitivity to weather, Tree Swallows are a particularly hardy and tractable study species (Jones 2003). They are secondary cavity nesters that will readily breed in artificial nest boxes and they are generally resistant to moderate levels of human disturbance.

Study Sites. This study took advantage of archival blood samples and historical nesting data collected from a long-established Tree Swallow nest box trail located in Tompkins County, New York, USA. During each breeding season (April-August), an observer recorded the contents of each nest box at least every 2-3 days until nestlings reached 12 days old, at which point checks became less frequent in order to avoid inducing premature fledging. Adult capture effort varied across study years depending on the needs and priorities of researchers, but in general, an effort was made to capture every breeding female using an observer-controlled nest box trap.

This effort was augmented early in the breeding season by non-targeted mist netting. Upon initial capture, each adult was banded with a USGS aluminum band bearing a unique identifying number. During subsequent captures, this same band number was read and recorded. In addition, a small blood sample was collected from each adult by brachial venipuncture and then stored in lysis buffer. Nestlings were similarly banded and bled between 7 and 14 days of age. These field methods resulted in a database that includes the presence and location of each individual in every year of study, as well as blood samples for a substantial fraction of the population. Because blood sampling did not become standard in our population until 2002, we only consider individuals born in 2002 or later.

Sample Selection. We were interested in following individual female recruits of known paternity status throughout their lives. Thus, we selected for inclusion in our study individuals who met the following criteria: (1) the individual must have been born and banded on one of our study sites between 2002 and 2011; (2) the individual must have returned to one of our study sites as an adult prior to 2014; (3) the individual's social parents must both have been captured; and (4) blood samples must have been collected for the focal individual and both social parents. We further excluded from our pool of female recruits those whose natal nests had received substantial disturbance as a result of ongoing experiments. We defined substantial disturbance as an experimental treatment that was expected to alter the nest environment in a sustained way (e.g. manipulation of brood size, nest microclimate, or bacterial load). Females from nests that received less intrusive experimental treatments (e.g. additional sampling or short-term trials of adults or nestlings) were allowed to remain.

Paternity Analysis. We used the QIAGEN[®] DNeasy Blood & Tissue Kit to extract genomic DNA from whole blood stored in lysis buffer. We used multiplex polymerase chain reaction (PCR) to amplify nine microsatellite loci previously developed to assess parentage in this species (Makarewich et al. 2009). Each forward primer was labeled with one of four

fluorescent dyes, and master mixes were devised such that each locus could be unambiguously identified by size range or color. PCR conditions were as follows: an initial denaturation step of 2 min at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 60 s of annealing at either 56°C or 58°C, and 60 s of extension at 72°C. A final elongation step was thereafter performed for 30 min at 72°C. PCR products were analyzed on an ABI 3730 x1 capillary sequencer, and Geneious (v.9.0.5; Kearse et al. 2012) was used to assign alleles.

We used CERVUS (v. 3.0; Kalinowski et al. 2007) to assign genetic sires to nestlings. Because our sample set of candidate males was restricted to the social fathers of our female recruits, we did not expect to identify extra-pair sires. Instead, we sought to determine whether the identified social father of each recruit was also her genetic sire. We assumed that each recruit's social mother was her genetic mother. Single locus mismatches of the social mother to the focal recruit were treated as genotyping error, while mismatches that occurred at more than one locus were treated as instances of sample misidentification. We identified a recruit as a WPO if (1) CERVUS designated her social father as her genetic father with greater than 95% confidence; or if (2) CERVUS designated her social father as the most likely sire with less than 95% confidence, but the social father's genotype was consistent with hers (i.e. one or fewer mismatched loci). All other recruits were treated as EPO.

We also used CERVUS to test for deviations from Hardy-Weinberg Equilibrium (HWE) and the presence of null alleles. Loci that did not conform to HWE expectations were removed from subsequent analyses.

Fitness Metrics. We measured six important life history traits that collectively influence the fitness of female Tree Swallows. First, we examined natal dispersal and breeding dispersal within our study area. Natal dispersal is defined as the movement of a young individual to its site of first breeding, while breeding dispersal is defined as subsequent movement from one site of breeding to another. The distance an individual disperses may carry with it significant fitness consequences. Individuals that disperse short distances may benefit from breeding at a site with

which they are already familiar and which has already proven to be adequate for rearing offspring (Yoder et al. 2004, Hoogland et al. 2006). On the other hand, individuals that leave their natal or prior breeding site may encounter reduced competition from both kin and non-kin or be better able to escape spatially-restricted stressors such as predation or low food abundance (e.g. Moore et al. 2006, Aguilon and Duckworth 2015). Decisions about whether and how to disperse may be influenced by proximal cues that serve as indicators of site quality, but they may also be influenced by an individual's personality: some individuals may simply be more dispersive than others (e.g. Cote and Clobert 2007, Duckworth 2008). From an individual standpoint, dispersal is important because of its potential to impact fitness (Hansson et al. 2004). From an analytical standpoint, dispersal is important because it directly influences estimates of other life history parameters. Individuals that disperse outside of a monitored study area may be incorrectly assumed to be dead, negatively biasing estimates of survival and lifetime reproductive success. Thus, it is important to understand whether dispersal varies among groups of interest.

We were unable to track individuals that dispersed outside of our study area, rendering measurements of true dispersal impossible. But by measuring the movements of short-distance dispersers who remained within our study area, we hoped to gain insight into whether EPO and WPO differ more broadly in their propensity to move away from a familiar site. We calculated natal dispersal as the distance between each individual's natal nest box and the first nest box in which it settled to breed. Breeding dispersal was likewise calculated as the distance between nest boxes used for breeding in two successive years. Nest box locations were gleaned from existing GPS coordinates associated with each box in our study area, and orthodromic distance was calculated using an online calculator that employs the Great Circle Method (<https://www.fcc.gov/media/radio/distance-and-azimuths>). Because natal dispersal tends to occur at longer distances than breeding dispersal and may entail different benefits and costs (Winkler et al. 2004, 2005), we chose to partition dispersal distances according to life stage.

We measured annual reproductive success as the number of offspring a female recruit

fledged during a single breeding attempt. Because swallows are generally single-brooded except in cases of nest failure, this usually amounted to a female's entire reproductive output during a given breeding season. Tree Swallow reproduction is known to be influenced by a number of intrinsic and extrinsic factors, including female age, clutch initiation date, and ambient temperature during breeding (reviewed in Winkler et al. 2011). In many populations, second-year (SY) females breeding for the first time have lower reproductive success than experienced after-second-year (ASY) females, as do females breeding later in the spring. Additionally, females subjected to prolonged periods of cold weather may suffer reproductive losses, especially if cold weather coincides with nestling rearing (Winkler et al. 2013). Even females who successfully fledge the same number of offspring may fledge them in very different conditions as a result of these challenges. And so, in addition to measuring reproductive output, we also measured average mass of surviving nestlings prior to fledging. Greater mass is associated with an increased probability of recruitment in our population (this thesis, Chapter 2) and so should provide a reliable measure of offspring quality. As in selection of female recruits, we eliminated from analysis any nesting attempt in which an experimental manipulation might reasonably have influenced productivity. Thus, nests subjected to brood, temperature, bacterial, or predator manipulation were excluded from our study.

In order to distinguish whether survival rates differed between EPO and WPO in our population, we measured the minimum lifespan of each recruit. Up to 75% of fledgling Tree Swallows are thought to perish in the first year of life, but adults typically experience annual mortality of only ~50%. This results in an average lifespan of approximately 2.5 years (reviewed in Winkler et al. 2011). Because all recruits were banded as nestlings on our sites, we knew with precision how old each adult was, even in cases where recruits delayed breeding for one or more years. Thus, we defined minimum lifespan as the age of the individual at its last capture. All individuals were followed through 2015. Individuals that endured experimental treatments in one or more years remained in the analysis unless the treatment resulted in total nest failure. Because reproductive failure might reasonably impact the likelihood of the individual returning to our site

to breed (Lima 2009, Schaub and von Hirschheydt 2009, Pakanen et al. 2011), we excluded such individuals from analysis.

Finally, we measured lifetime reproductive success as the total number of fledglings produced by each female recruit over the course of her life. This measure integrates all of the other metrics recorded for these birds and provides the most holistic measure of recruit fitness. Due to experimental manipulations at some recruit nests, we were unable to specify the number of fledglings from every nest in every year. When reproductive success was unavailable due to manipulation, we assumed that the recruit would have produced the average number of fledglings she produced in other years during which her nest was undisturbed. We excluded from analysis individuals for whom we had no information on reproduction, as well as those individuals for whom at least one nest was experimentally destroyed.

Environmental Metrics. In order to assess environmental stress, we quantified temperatures that recruits encountered each year at our sites. We partitioned temperature variation according to conditions that recruits experienced as nestlings (hereafter, HY (hatch-year) temperatures) and those they experienced as breeding adults (hereafter, AHY (after-hatch-year) temperatures). All temperature data were retrieved from the Game Farm Road Weather Station in Ithaca, NY (<http://www.nrcc.cornell.edu/wxstation/ithaca/ithaca.html>).

For nestlings, we assumed that the most relevant temperatures were those experienced during the nesting cycle. And so, we calculated temperature metrics for each nestling recruit during each of three natal phases: (1) incubation, (2) early nestling period, and (3) late nestling period. Incubation was assumed to begin on the day the last egg of the clutch was laid and to conclude on the day before hatching. Because Tree Swallows typically lay one egg each day until their clutch is complete, we had very reliable data on when a clutch had been completed (as we often checked nest boxes during laying). However, hatching is fairly synchronous in Tree Swallows, often occurring on a single day, and boxes were not always checked daily. In cases where there was some uncertainty surrounding timing of hatching, we assumed a 14-day

incubation period. If a 14-day incubation period was inconsistent with nest observations (e.g. if nestlings were observed between 11 and 13 days post-clutch completion), we adopted the hatch date closest to a 14-day incubation period as the estimated hatch date. For the early nestling period, we calculated temperature metrics during a period that began on the estimated or actual hatch date and concluded when nestlings were 8 days old. For the late nestling period, we calculated temperatures during nestling days 9-17. Tree Swallows typically fledge at 18-22 days old, so our three time periods collectively span natal experience from the onset of incubation until independence.

For each time period, we calculated two temperature metrics. First, we quantified average daily maximum temperature. Because Tree Swallows rely almost exclusively on flying insects to meet their dietary and provisioning needs during the breeding season, their food supply is tightly linked to ambient temperatures. In fact, widespread nestling mortality has been documented at sustained daily maximum temperatures below 18.5°C (Winkler et al. 2013). In addition to average temperatures, we sought a measure of temperature variability. More variable environmental conditions are generally assumed to be challenging because they necessarily place strain on homeostasis, but when encountered early in life, they may also have the positive effect of priming organisms to cope with environmental variability as an adult (Constantini et al. 2010). We quantified temperature variability as the average absolute swing in daily maximum temperature during each interval of interest. Thus, higher values in this metric signified maximum daily temperatures that vacillated more significantly through time. Because we were interested in evaluating each individual's experience relative to that of other individuals in the population, and did not know *a priori* what the shape of the relationship between temperature and fitness looked like, we elected to partition individuals into broad groups according to whether their environmental experience fell above or below the mean experience of all individuals in our population. We categorized average daily high temperatures as either 'cold' or 'warm' compared to the mean temperature conditions experienced by all individuals in our study at each stage of life. We likewise categorized temperature variability as either 'high' or 'low'

(Table 3.1a).

We used these same metrics to characterize AHY temperatures, but expanded our time intervals of interest to more fully encompass environmental experience from an adult perspective. Tree Swallows are one of the earliest songbirds to return to their breeding grounds in the spring, often arriving at northern latitudes while snow is still on the ground (reviewed in Winkler et al. 2011). This perhaps gives them an advantage in competing for scarce nesting sites, but it carries the potentially severe cost of encountering adverse, sometimes fatal, temperatures (e.g. Hess et al. 2008). We calculated average daily maximum temperature and average temperature swing during the months of March and April in each year in order to characterize pre-breeding weather conditions experienced by adult recruits. We likewise quantified both temperature metrics during the months of May and June to characterize conditions experienced during breeding. Because these temperature metrics were not unique to an individual, but instead represented a set of conditions experienced by all individuals breeding in a given year, we did not consider these as individual covariates. Rather, for each time period (pre-breeding and breeding), we characterized each year from 2003-2015 as being warm or cold relative to the average temperature observed across all other years of study. We likewise characterized each year as being of high or low variability (Table 3.1b).

Table 3.1: (a) Environmental metrics used in models of life history of female recruits. Six metrics (labeled ‘HY’ for ‘hatch-year’) were used to characterize the natal environment experienced by each recruit during each of three phases of development (Inc = incubation, N1= early nestling, N2 = late nestling). In each phase of development, we calculated average temperature (T) and temperature variability (Var). These values were used to partition each recruit’s experience into “cold” or “warm” average temperatures and “high” or “low” temperature variability with reference to the mean values of all recruits. Four metrics (labeled ‘AHY’ for ‘after-hatch-year’) were used to characterize weather conditions experienced by recruits as adults during pre-breeding (PB) and breeding (B) seasons. (b) Average PB and B temperature and temperature variability during each year of study. Red indicates years that were considered ‘warm’, while blue indicates years that were considered ‘cold’. Purple indicates years that were considered to exhibit ‘high’ temperature variability, and green indicates years considered to have ‘low’ temperature variability. For ‘B Ave High T’, we also used the number of cold snap days (maximum temperature below 18.5°C; Winkler et al. 2013) to help inform decisions about adult experience. The number of cold snap days is given in parentheses.

(a)

Environmental Metric	Time Period	Description	Definitions
HY Inc T	Day last egg laid – day prior to hatch	Average daily high temperature	16.8-22.8°C = “Cold” 22.8-28.8°C = “Warm”
HY N1 T	Nestling days 0-8	Average daily high temperature	19.1-24.6°C = “Cold” 24.6-29.4°C = “Warm”
HY N2 T	Nestling days 9-17	Average daily high temperature	21.6-25.1°C = “Cold” 25.1-30.4°C = “Warm”
HY Inc Var	Day last egg laid – day prior to hatch	Average absolute difference between daily high temperatures	1.8-3.3°C = “Low” 3.3-5.1°C = “High”
HY N1 Var	Nestling days 0-8	Average absolute difference between daily high temperatures	1.8-3.3°C = “Low” 3.3-5.4°C = “High”
HY N2 Var	Nestling days 9-17	Average absolute difference between daily high temperatures	1.5-3.1°C = “Low” 3.1-5.1°C = “High”
AHY PB T	Current March-April	Average daily high temperature	See Table 1b
AHY B T	Current May-June	Average daily high temperature	See Table 1b
AHY PB Var	Current March-April	Average absolute difference between daily high temperatures	See Table 1b
AHY B Var	Current May-June	Average absolute difference between daily high temperatures	See Table 1b

Table 3.1 (Continued)

(b)

Year	AHY PB T (°C)	AHY PB Var (°C)	AHY B T (°C)	AHY B Var (°C)
2003	9.2	6.4	20.4 (23)	4.0
2004	9.9	4.0	22.4 (13)	4.0
2005	8.8	3.9	22.3 (19)	2.9
2006	9.4	3.8	21.5 (19)	3.2
2007	7.7	4.8	23.6 (13)	3.8
2008	10.3	4.3	21.9 (21)	3.4
2009	11.0	5.6	21.4 (14)	2.9
2010	12.9	4.3	23.2 (11)	3.6
2011	8.5	4.8	22.6 (13)	3.5
2012	13.1	5.3	24.1 (10)	3.3
2013	8.0	3.5	22.2 (15)	3.8
2014	7.0	6.0	22.2 (16)	3.7
2015	7.0	5.2	23.3 (11)	3.9

Statistical Analyses. We used linear mixed effects models (*lmer* or *glmer* functions in the lme4 package in R v.3.2.0) to evaluate the relative support for models representing each of our hypotheses concerning the individual and interactive effects of extra-pair status and environment on six life history traits. Each set of candidate models consisted of a null model, a model in which the effect of extra-pair status was independently tested, and a series of 6-10 models in which extra-pair status was tested in interaction with each environmental variable. We used Akaike's Information Criterion (AIC; Akaike 1973) to evaluate the relative support for each model. Because candidate models containing environmental metrics represented independent, rather than alternative, hypotheses, we did not rank models relative to one another. Instead, we compared each candidate model against the null, and considered any models which outperformed the null model by at least 2 points to be well-supported.

We used general linear mixed effects models with a Gamma distribution to model the effects of extra-pair status and environment on natal and breeding dispersal distance. Because the Gamma distribution cannot accommodate zeroes, but we had several individuals return to the same nest box in successive years, we added a nominal (0.001 km) transformation to every dispersal distance prior to analysis. In both sets of models, we included natal nest box as a

random effect. When modeling breeding dispersal distance, we also included recruit identity as a random effect. For natal dispersal distance, we tested the effect of natal nest site prior to running candidate models. Since our study sites are different sizes and distances from one another, it is possible that natal dispersal distance might vary predictably as a function of the availability of boxes at and proximity to other sites. However, a model including natal nest site as a random effect performed no better than a null model that did not include this term, and thus our simpler null model was retained. We considered extra-pair status and all HY temperature variables as fixed effects for both types of dispersal. For breeding dispersal distance, we additionally examined models in which dispersal varied as a function of AHY temperature and temperature variability in the previous year. For natal dispersal, no similar AHY effects were included since natal dispersal occurred prior to breeding experience as an adult.

We used linear mixed effects models to examine the influence of extra-pair status and environment on annual reproductive success. Natal nest box and recruit identity were included as random effects in all models. We additionally tested a number of fixed effects prior to building our candidate models in order to accommodate factors that might influence fledgling number or nestling mass, but which were otherwise outside the scope of our hypotheses. Thus, we tested the independent, additive, and interactive effects of Julian lay date (1 = May 1) and female age class (SY or ASY) on fledgling number, and the effects of Julian lay date, female age class, and age at measurement on average nestling mass. For fledgling number, none of these models performed significantly better than our existing null model (random effects only), but there was a strong positive effect of age at measurement on average nestling mass. Thus, for all candidate models designed to evaluate this life history metric, we included age of measurement as a fixed effect. For both fledgling number and nestling mass, we examined models which included independent and interactive effects of extra-pair status with all HY and AHY temperature variables.

We used general linear mixed effects models with a Poisson distribution to examine factors that might influence minimum lifespan of recruits. We -1 transformed all lifespans in order to accommodate the Poisson distribution's requirement of '0' values (since all recruits had,

by definition, survived to at least age 1, the original data were devoid of zeroes), and included natal nest box as a random effect in all models. Extra-pair status and all HY temperature variables were included as fixed effects. AHY temperature variables were ‘averaged’ over the course of each individual’s adult life, such that individuals who experienced a preponderance of ‘warm’ pre-breeding or breeding seasons were categorized as having a ‘warm’ adulthood, individuals who experienced a preponderance of ‘cold’ pre-breeding or breeding seasons were categorized as having a ‘cold’ adulthood, and individuals who experienced an equal number of warm and cold pre-breeding or breeding seasons were categorized as ‘mixed’. In the same way, we designated the pre-breeding and breeding temperature variability of each individual’s adulthood as either, ‘high’, ‘low’, or ‘mixed’. These categorizations were carried through the candidate model set as fixed effects.

Finally, we used general linear models with a Poisson distribution to examine the effects of extra-pair status and environment on lifetime reproductive success. In these models, we included natal nest box as a random effect, and extra-pair status and all HY temperature variables as fixed effects. We likewise included AHY temperature variables as fixed effects according to the definitions given for minimum lifespan. Thus, AHY temperature variables represented the average conditions experienced over an individual’s entire adulthood.

Results

Dispersal. Extra-pair status had no effect on natal or breeding dispersal distance, either independently or in interaction with any environmental metric we considered (Table 3.2).

Table 3.2: Full model selection for (a) natal dispersal distance and (b) breeding dispersal distance of female recruits. General linear mixed effects models with a gamma distribution were used to model dispersal distance, and each model included nest box of origin and recruit identity as random effects where appropriate. In each model set, the null model is shaded in green. Models that outperformed the null model by $\Delta AIC > 2$ are shaded in blue. For breeding dispersal distance, two models (EP Status*Last Year PB Var and EP Status*Last Year B T) were excluded from the candidate model set after the models failed to converge. k = number of estimated parameters, AIC = Akaike's Information Criterion (Akaike 1973), $EP\ Status$ = recruit extra-pair status, HY = hatch year, AHY = after hatch year, Inc = incubation, $N1$ = early nestling period, $N2$ = late nestling period, PB = pre-breeding, B = breeding, T = average temperature, Var = average temperature variability.

(a) Natal dispersal distance

Model	n	k	ΔAIC
EP Status*HY N2 Var	61	4	0
EP Status * HY Inc Var	61	4	1.12
EP Status	61	2	1.44
EP Status * HY N1 Var	61	4	1.81
Intercept	61	1	1.93
EP Status*HY N2 T	61	4	3.29
EP Status*HY N1 T	61	4	5
EP Status*HY Inc T	61	4	5.28

(b) Breeding dispersal distance

Model	n	k	ΔAIC
EP Status*HY N1 T	40	4	0
Intercept	40	1	1.85
EP Status	40	2	3.79
EP Status*Last Year B Var	40	4	4.72
EP Status*HY Inc Var	40	4	5.14
EP Status*HY Inc T	40	4	5.27
EP Status*HY N1 Var	40	4	5.37
EP Status*Last Year PB T	40	4	5.54
EP Status*HY N2 Var	40	4	7.15
EP Status*HY N2 T	40	4	7.21

Annual Reproductive Success. While there was no independent effect of extra-pair status on annual reproductive success, three supported models indicated that an interaction between environment and extra-pair status influenced fledgling production (Table 3.3a, Figure 3.1). Specifically, the effect of extra-pair status on fledgling production depended on average temperatures experienced by recruits during the late nestling period ($p = 0.10$), average temperatures during breeding ($p = 0.006$), and temperature variability during breeding ($p = 0.04$).

Post-hoc pairwise comparisons revealed that WPO reared in warm temperatures produced more fledglings per breeding attempt than either EPO ($p = 0.08$) or WPO ($p = 0.03$) reared in cold temperatures. EPO who bred in cold temperatures produced significantly more fledglings than EPO who bred in warm temperatures ($p = 0.03$). And WPO who bred in variable temperatures tended to outperform WPO who bred under more constant conditions ($p = 0.09$).

For average nestling mass, though our best-supported model included a significant interaction between pre-breeding temperature variability and extra-pair status, neither the interaction term nor any of its pairwise comparisons indicated any differences between EPO and WPO (Table 3.3b).

Table 3.3: Full model selection for recruit reproductive success: (a) number of fledglings produced per breeding attempt and (b) average nestling mass. Linear mixed effects models included nest box of origin and recruit identity as random effects. In each model set, the null model is shaded in green. Models that outperformed the null model by $\Delta AIC > 2$ are shaded in blue. k = number of estimated parameters, AIC = Akaike's Information Criterion (Akaike 1973), $EP\ Status$ = recruit extra-pair status, HY = hatch year, AHY = after hatch year, Inc = incubation, $N1$ = early nestling period, $N2$ = late nestling period, PB = pre-breeding, B = breeding, T = average temperature, Var = average temperature variability.

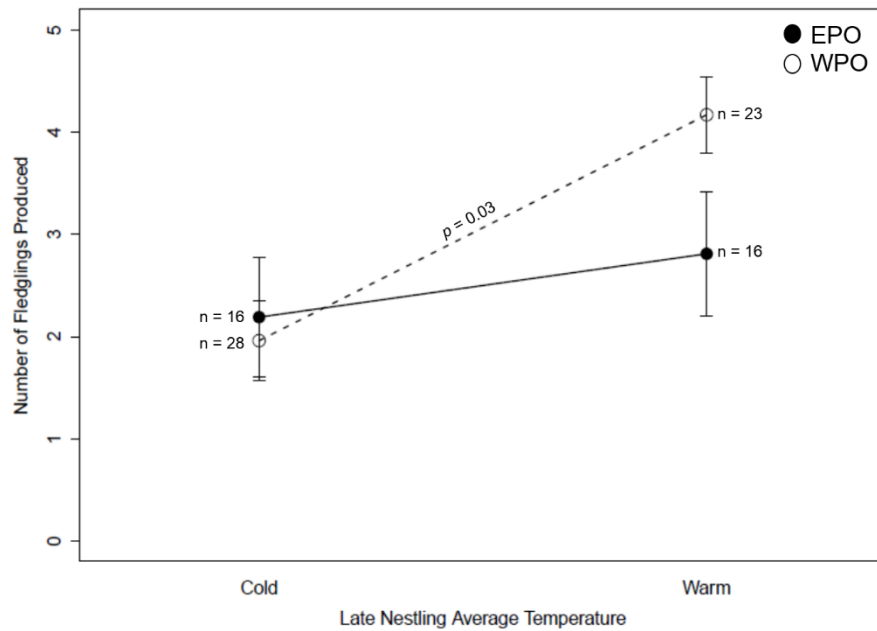
(a) Number of nestlings fledged per breeding attempt

Model	n	k	ΔAIC
EP Status*HY N2 T	83	4	0
EP Status*AHY B T	83	4	4.41
EP Status*AHY B Var	83	4	5.78
EP Status*HY N1 Var	83	4	8.28
EP Status*HY N2 Var	83	4	8.43
EP Status*AHY PB T	83	4	9.78
Intercept	83	1	9.92
EP Status*AHY PB Var	83	4	10.44
EP Status	83	2	10.81
EP Status*HY Inc T	83	4	11.54
EP Status*HY Inc Var	83	4	11.88
EP Status*HY N1 T	83	4	11.92

(b) Average nestling mass

Model	n	k	ΔAIC
EP Status*AHY PB Var + Age Measured	45	5	0
EP Status*HY Inc Var + Age Measured	45	5	1.44
EP Status*HY N1 Var + Age Measured	45	5	1.74
EP Status*AHY B T + Age Measured	45	5	1.87
EP Status*HY N2 T + Age Measured	45	5	2.24
EP Status*AHY B Var + Age Measured	45	5	2.29
EP Status*HY Inc T + Age Measured	45	5	2.67
EP Status*HY N2 Var + Age Measured	45	5	2.95
EP Status*AHY PB T + Age Measured	45	5	3.03
Age Measured	45	2	3.05
EP Status*HY N1 T + Age Measured	45	5	3.21
EP Status + Age Measured	45	3	3.62

(a)



(b)

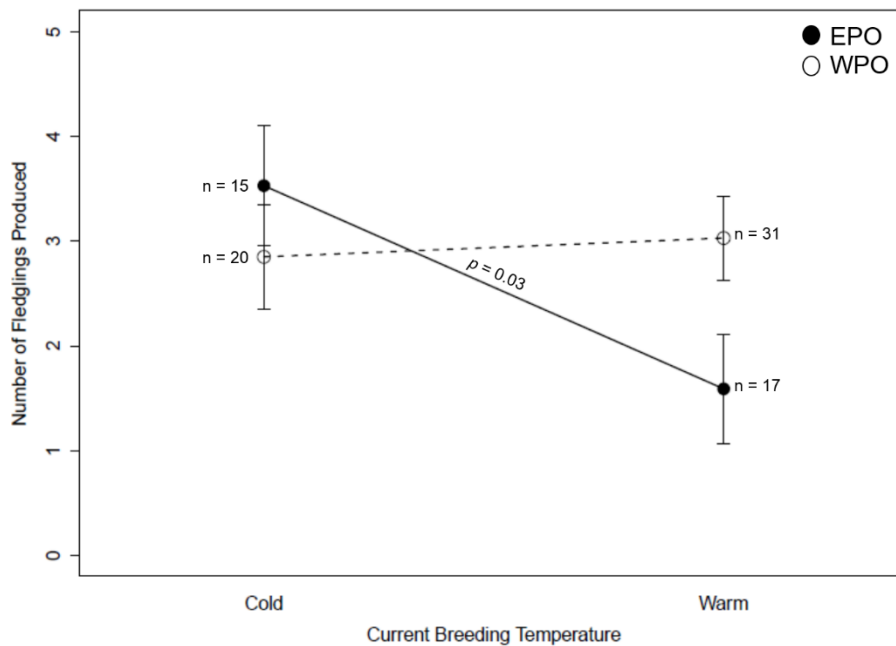
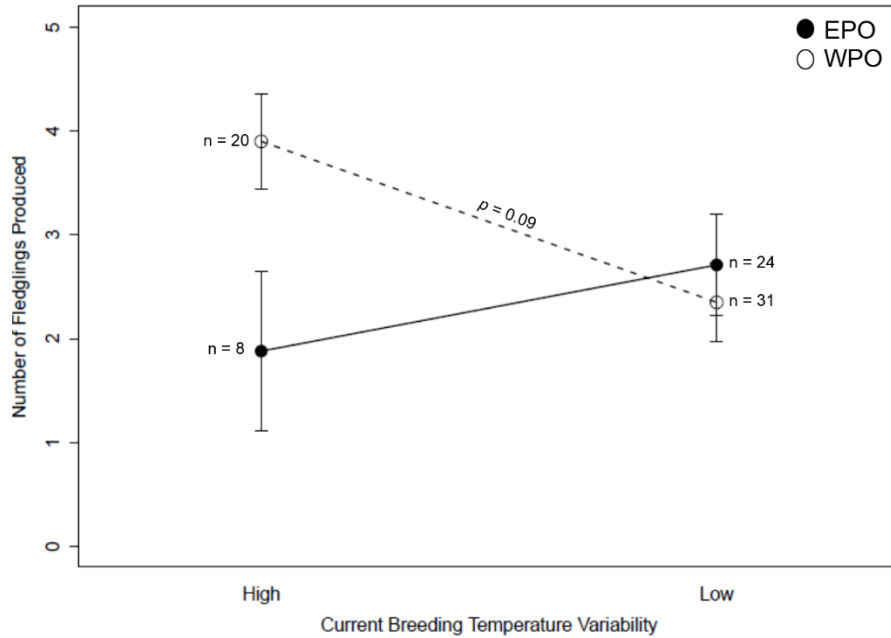


Figure 3.1: Interactive effects of extra-pair status and (a) average temperatures during the late nestling period, (b) average temperatures during the current breeding season, and (c) temperature variability during the current breeding season on fledgling production in female recruits. Plotted points represent means \pm SE for each group. Sample sizes are indicated next to each point. Significant pairwise differences are indicated by p-values.

Figure 3.1 (Continued)

(c)



Lifespan. Our analysis of lifespan produced a single model that received support over our null, and this indicated a significant interaction between extra-pair status and temperature variability during the late nestling period ($p = 0.03$; Table 3.4). WPO reared in less variable temperatures survived significantly longer than either WPO reared in more variable conditions ($p = 0.03$) or EPO reared in less variable conditions ($p = 0.08$; Figure 3.2).

Table 3.4: Full model selection for recruit minimum lifespan. General linear mixed effects models with a Poisson distribution were used to model lifespan, and each model included nest box of origin as a random effect. In each model set, the null model is shaded in green. Models that outperformed the null model by $\Delta AIC > 2$ are shaded in blue. k = number of estimated parameters, AIC = Akaike's Information Criterion (Akaike 1973), EP Status = recruit extra-pair status, HY = hatch year, AHY = after hatch year, Inc = incubation, $N1$ = early nestling period, $N2$ = late nestling period, PB = pre-breeding, B = breeding, T = average temperature, Var = average temperature variability.

Model	n	k	ΔAIC
EP Status*HY N2 Var	61	4	0
Intercept	61	1	3.97
EP Status	61	2	4.75
EP Status*HY N2 T	61	4	4.78
EP Status*HY Inc Var	61	4	6.32
EP Status*HY N1 Var	61	4	6.58
EP Status*HY N1 T	61	4	6.72
EP Status*HY Inc T	61	4	7.34
EP Status*AHY PB Var	61	5	7.75
EP Status*AHY PB T	61	5	8.04
EP Status*AHY B T	61	5	8.12
EP Status*AHY B Var	61	5	10.02

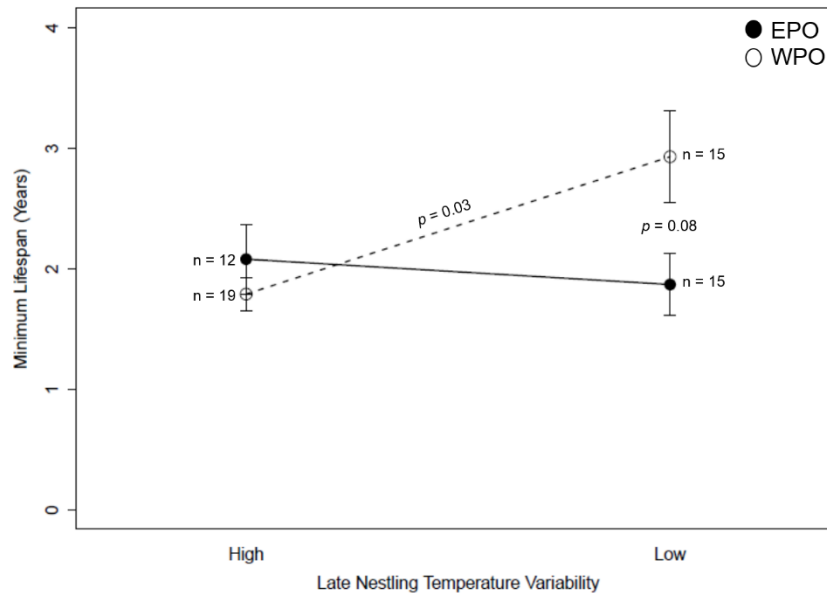


Figure 3.2: Interactive effect of extra-pair status and temperature variability during the late nestling period on minimum lifespan of female recruits. Plotted points represent means \pm SE for each group. Sample sizes are indicated next to each point. Significant pairwise differences are indicated by p-values.

Lifetime Reproductive Success. Four candidate models performed better than the null model in our analysis of factors influencing lifetime reproductive success (Table 3.5). These models indicated that lifetime reproductive success depended on interactions between extra-pair status and (1) temperature variability during incubation ($p = 0.01$), (2) temperature variability during the early nestling period ($p = 0.008$), (3) average temperatures during the late nestling period ($p = 0.05$), and (4) temperature variability during the late nestling period ($p = 0.09$). Pairwise comparisons between EPO and WPO for each of these models (Figure 3.3) revealed that (1) WPO who had experienced more variable ambient temperatures while being incubated produced significantly more fledglings in their lifetime than EPO incubated under similar conditions ($p = 0.04$) and tended to produce more fledglings than WPO incubated in less variable ambient temperatures ($p = 0.06$); (2) WPO who had experienced less variable temperatures during the early nestling period produced significantly more fledglings in their lifetime than either EPO reared under similar conditions ($p = 0.03$) or WPO reared under more variable conditions ($p = 0.04$); (3) WPO reared in warmer temperatures during the late nestling period produced more lifetime fledglings than EPO (warm: $p = 0.06$, cold: $p = 0.02$) or WPO reared in colder temperatures ($p = 0.01$); and (4) WPO reared in less variable temperatures during the late nestling period produced more lifetime fledglings than EPO (high variability: $p = 0.05$, low variability: $p = 0.10$) or WPO reared in more variable temperatures ($p = 0.03$).

Table 3.5: Full model selection for recruit lifetime reproductive success. General linear mixed effects models with a Poisson distribution were used to model lifetime number of fledglings, and each model included nest box of origin as a random effect. In each model set, the null model is shaded in green. Models that outperformed the null model by $\Delta AIC > 2$ are shaded in blue. k = number of estimated parameters, AIC = Akaike's Information Criterion (Akaike 1973), EP Status = recruit extra-pair status, HY = hatch year, AHY = after hatch year, Inc = incubation, $N1$ = early nestling period, $N2$ = late nestling period, PB = pre-breeding, B = breeding, T = average temperature, Var = average temperature variability.

Model	n	k	ΔAIC
EP Status*HY N2 T	49	4	0
EP Status*HY N1 Var	49	4	0.29
EP Status*HY Inc Var	49	4	1.45
EP Status*HY N2 Var	49	4	2.21
Intercept	49	1	4.26
EP Status	49	2	5.27
EP Status*HY Inc T	49	4	5.96
EP Status*AHY PB T	49	5	6.05
EP Status*HY N1 T	49	4	7.99
EP Status*AHY B Var	49	5	8.24
EP Status*AHY B T	49	5	9.55
EP Status*AHY PB Var	49	5	10.87

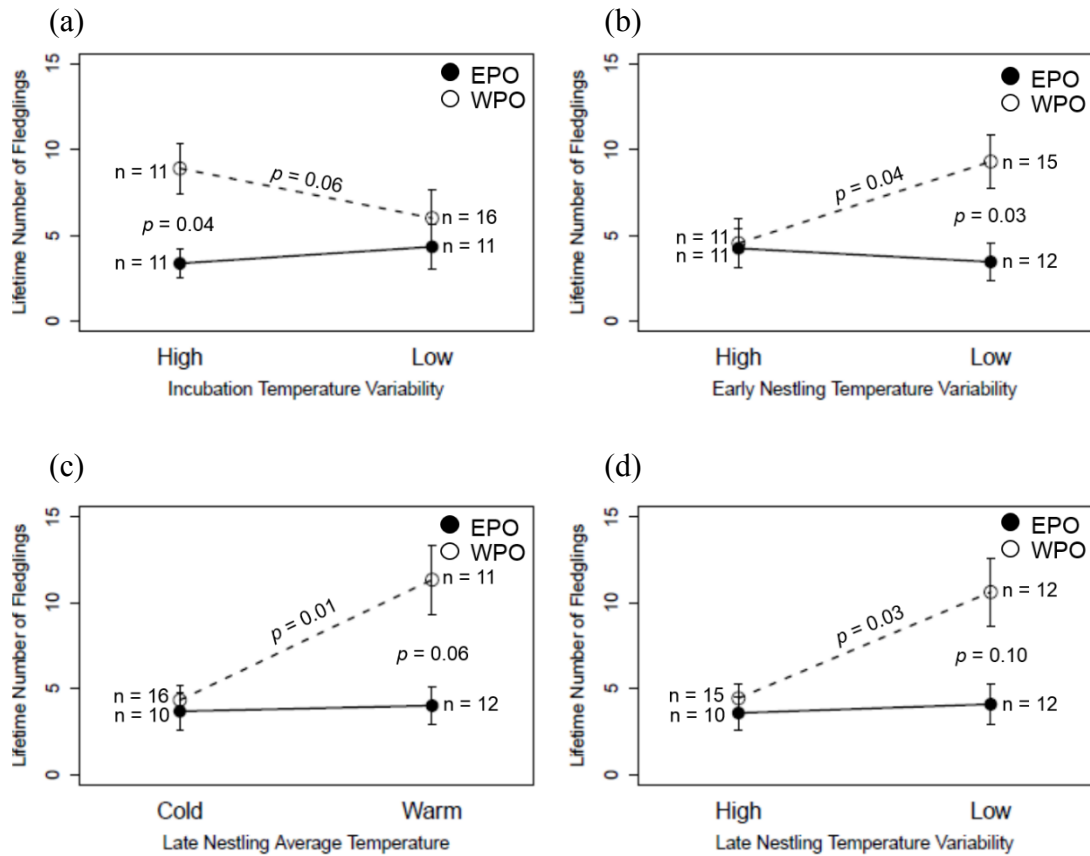


Figure 3.3: Interactive effects of extra-pair status and (a) temperature variability during incubation, (b) temperature variability during the early nestling period, (c) average temperature during the late nestling period, and (d) temperature variability during the late nestling period on minimum lifetime reproductive success of female recruits. Plotted points represent means \pm SE for each group. Sample sizes are indicated next to each point. Significant pairwise differences are indicated by p-values.

Discussion

EPP is perhaps one of the best-studied topics in avian ecology, but benefits to females of mating outside of the pair bond have proved elusive. One of the most frequently examined hypotheses – the genetic benefits hypothesis – posits that females mate multiply in order to secure better genes for their offspring. But its chief prediction – that EPO should exhibit higher fitness than WPO – has been met in fewer than 50% of published studies (Akçay and Roughgarden 2007). One possible explanation for this lack of consensus is that benefits of EPP depend on environmental context (Schmoll 2011). EPO may perform better in some

circumstances than in others, providing a benefit to females who mate multiply when such conditions arise. Another explanation is that genetic benefits only become apparent at advanced stages of life, long after fledglings have left the nest. As most studies of EPP have only looked for fitness differences prior to fledging, they may have missed fitness differences that emerge later on.

In this study, we sought to simultaneously address both of these deficiencies by examining fitness variation in female Tree Swallow recruits of known paternity status. We investigated whether EPO and WPO recruits varied in six important life history traits, and whether variation in life history depended on environmental conditions encountered by recruits during development or as adults. We found no overall effect of extra-pair status on any of the life history traits that we measured. However, when we allowed life history traits to vary by extra-pair status *and* environment, we found several traits in which EPO and WPO diverged. We were principally interested in comparing EPO and WPO within specific environmental contexts (e.g. in cold or warm temperatures). However, our analysis also detected a number of differences between nestlings of one paternity status (e.g. WPO) across environmental contexts (e.g. cold temperatures vs. warm temperatures). It is important to distinguish between differences that occur within members of one group (EPO or WPO) across environmental contexts, and those that occur across groups (EPO vs. WPO) within the same environmental context (Table 3.6) because they represent fundamentally different things. We consider each of these scenarios in turn.

Phenotypic plasticity is typically used to describe the potential of a single genotype to produce multiple phenotypes, but it can also refer more broadly to the ability of an individual or group of individuals to enlist different phenotypic responses depending on the environmental conditions they encounter (reviewed in Whitman and Agrawal 2009). Plasticity falls broadly into two types. First, environmental variation during development can permanently set an individual's phenotype on a particular course, thereby limiting the plasticity of the individual, but fostering a series of alternative phenotypes in the population as a whole. Examples of this

type of plasticity (hereafter, ‘developmental plasticity’) include density-induced personality variation in Western Bluebirds (*Sialia mexicana*; Duckworth 2008), predator-induced morphological variation in tadpoles of the Striped Marsh Frog (*Limnodynastes peronii*; Kraft et al. 2006), or life history shifts that occur in larval Long-toed Salamanders (*Ambystoma macrodactylum*) in the presence of cannibalistic conspecifics (Wildy et al. 1999). Plasticity can also vary within individuals, such that some individuals are highly responsive to changes in their environment (high plasticity) and other individuals behave consistently across environmental contexts (low plasticity). Examples of this type of plasticity (hereafter, ‘individual plasticity’) include the ability of Great Tits (*Parus major*) to adjust laying date in response to warming spring temperatures (Charmantier et al. 2008), rapid adjustment of song performance by male Common Yellowthroats depending on social context (Taff et al. 2016), and induction of any number of physiological pathways in response to environmental stress. Plasticity of either type may be beneficial in allowing organisms to optimize responses to a variable and unpredictable environment, but it can also carry significant developmental and metabolic costs (DeWitt et al. 1998). Whether plasticity is advantageous should thus depend on the stability and predictability of a particular environment.

Table 3.6: Summary of significant and marginally non-significant ($0.05 < p < 0.10$, indicated by *) pairwise differences observed among female recruits in six life history metrics. Differences are color-coded according to whether the environmental metric of interest affected individuals during development (red) or adulthood (blue). *NDD* = natal dispersal distance, *BDD* = breeding dispersal distance, *LRS* = lifetime reproductive success, *WPO* = within-pair offspring, *EPO* = extra-pair offspring, *INC* = incubation, *NI* = early nestling period, *N2* = late nestling period, *B* = breeding season, *w* = warm, *c* = cold, *var* = high variability, *con* = low variability.

Life History Trait	Plasticity (WPO)	Plasticity (EPO)	Genetic Benefits	Context-dependent Genetic Benefits
NDD				
BDD				
Reproduction	$WPO_{N2w} > WPO_{N2c}$ $WPO_{Bvar} > WPO_{Bcon}^*$	$EPO_{Bc} > EPO_{Bw}$		
Nestling Mass				
Lifespan	$WPO_{N2con} > WPO_{N2var}$			$WPO_{N2con} > EPO_{N2con}$
LRS	$WPO_{INCvar} > WPO_{INCcon}^*$ $WPO_{N1con} > WPO_{N1var}$ $WPO_{N2w} > WPO_{N2c}$ $WPO_{N2con} > WPO_{N2var}$			$WPO_{INCvar} > EPO_{INCvar}$ $WPO_{N1con} > EPO_{N1con}$ $WPO_{N2w} > EPO_{N2w}^*$ $WPO_{N2con} > EPO_{N2con}^*$

Our results suggest that several Tree Swallow life history traits exhibit plasticity in response to environmental variation. Two important patterns emerge. First, most of the plasticity that we observed was related to variation in developmental environment rather than contemporary conditions that recruits experienced as adults (Table 3.6). Developmental environment has been shown to have a profound and lasting effect on adult phenotypes in many species, and can result in alternative morphologies, physiologies, and behaviors (reviewed in Whitman and Agrawal 2009). But for a species whose ecology is so tightly coupled to contemporary environmental conditions to show such a strong and near-universal dependence on developmental environment is surprising. Reproduction, survival, and lifetime reproductive success all reflected conditions that recruits had encountered as nestlings. Only annual reproductive success showed any dependence on conditions experienced by adults.

Second, plasticity was, by and large, restricted to WPO (Table 3.6). Only in a single metric (annual reproductive success) did we observe evidence of life history plasticity within EPO, and in this one instance, it was contemporary environment that mediated it. Thus, WPO were substantially more developmentally plastic than their extra-pair counterparts.

This is significant because it is this plasticity that accounts for the superior performance of WPO under certain environmental conditions. Whenever we observed an absolute fitness difference between EPO and WPO, it always coincided with a fitness difference between WPO reared in different environments. As a group, WPO were no better than EPO in any of the metrics we considered. But among recruits incubated in more variable temperatures, and reared in warmer and less variable temperatures, those that were within-pair survived longer and produced significantly more fledglings in their lifetimes than those of extra-pair origin. In contrast, EPO never outperformed WPO in any of the metrics we considered. In every instance, in every environment, they performed the same or worse.

At the outset of this study, we hypothesized that EPO were of intrinsically higher quality than WPO, and that they would therefore exhibit higher fitness in certain environmental contexts. Specifically, we predicted that genetic benefits of EPP might be revealed when conditions were stressful. Instead, we found that WPO tended to outperform EPO as adults when environmental conditions during development were more benign (warmer and less variable).

In one sense, our results are consistent with those of other studies that have reported WPO to have higher fitness than EPO (e.g. Sardell et al. 2011, Hsu et al. 2014), and could be similarly interpreted as providing evidence that extra-pair mating imposes costs on females. After all, we found that EPO performed similarly to or worse than WPO in every fitness metric that we measured and across all environmental contexts. However, this hypothesis does not offer any obvious explanation for why WPO should be more developmentally plastic than EPO, unless loss of offspring plasticity is the cost that females pay for mating multiply. This is certainly possible if, as suggested by Hsu et al. (2014), extra-pair sires are passing lower-quality genetic material onto their offspring, but further research is needed to determine whether this is the case. In addition, it is not intuitive why genetically superior WPO would perform best under relatively benign, as opposed to poor, conditions.

Alternatively, our results may reflect a subtle benefit of promiscuity: the nestlings resulting from such matings may be more tolerant of environmental stress. Under this

hypothesis, the advantage of producing EPO comes primarily from their life history stability – regardless of natal conditions, they are likely to produce a safe, low rate of fitness return. In contrast, the advantage of producing WPO comes from their life history flexibility – they are able to perform exceptionally well when environmental conditions are favorable. However, should natal conditions prove challenging, their heightened sensitivity to natal environment means that exceptionally harsh conditions may produce exceptionally poor offspring. The appeal of this hypothesis lies in its ability to explain both why EPO and WPO differ in some environmental contexts, but not others, and why WPO should have higher fitness when environmental conditions are benign.

However, this hypothesis depends on the existence of environmental contexts in which EPO outperform WPO, but these were never observed. This could have occurred because we did not encounter conditions that were stressful enough to result in higher EPO fitness during our study period, or because the way in which we binned our environmental variables was too broad to capture them. The generally small sample size of genotyped recruits precluded a thorough comparison of individuals who experienced the most extreme developmental conditions, but a more detailed study of offspring fitness outcomes across a variety of contexts would be helpful.

Interestingly, the low developmental plasticity of EPO life history may result from a high degree of individual plasticity. If the environment pushes an individual out of homeostasis, there are two possible outcomes: (1) the individual changes in concert with the environment such that a new homeostatic balance is reached; or (2) the individual pushes itself back into original homeostasis (reviewed in Whitman and Agrawal 2009). We suggest that WPO might employ the first strategy, and EPO, the second. If this is the case, we would expect to see physiological symptoms of greater individual plasticity in EPO in response to stress. We have no data on the physiological resiliency of EPO and WPO in our population, but studies demonstrating higher immunocompetence of EPO reared in stressful developmental contexts (Garvin et al. 2006, Arct et al. 2013) are consistent with this hypothesis.

It is interesting to consider mechanisms that might have resulted in nestlings of one

paternity status exhibiting greater developmental plasticity than the other. While we have no data that might explain this phenomenon, we can suggest several possibilities. First, females may select as extra-pair mates males who are more resistant to environmental perturbation. Extra-pair mating is expected to result in a number of unique costs, including increased probability of disease transmission, increased energetic and temporal expenditure as a result of searching for potential mates, increased frequency of agonistic encounters with resident males, and loss of paternity at the social nest. The ability to navigate these stressors successfully may make for a particularly hardy sire that produces similarly hardy (if low-fitness) offspring. In fact, epigenetic paternal effects may help to explain how differences between WPO and EPO arise, even when many males take on the dual role of within-pair and extra-pair sire. DNA methylation patterns have been shown to vary with age (Jenkins et al. 2014), and age is one of the most consistent predictors of extra-pair fertilization success (reviewed in Akçay and Roughgarden 2007). Recently, it was discovered that DNA methylation patterns in sperm can also vary as a result of chronic paternal stress (Rodgers et al. 2015). Thus, older or more stressed males may produce fundamentally different types of offspring than younger or less stressed males, independent of other aspects of their quality. Females also have the potential for tremendous influence on nestling development, and can foster variation between offspring by adjusting deposition of antibodies, growth factors, or hormones into eggs (e.g. Bentz et al. 2013, Duckworth et al. 2015), or by altering laying order or sex of offspring (Krist et al. 2005, Vedder et al. 2013). In addition to genetic, epigenetic, or maternal effects cultivating differences between WPO and EPO, nestlings of differing paternity status could also vary in plasticity as a result of systematic variation in their natal environment. Though siblings from mixed-paternity broods are reared in the same nest, WPO are reared by the male who sired them, while EPO are reared by an unrelated father. If social males provide parental care in a way that better accommodates offspring bearing their genes (e.g. if a male provisions offspring less and also produces offspring genetically disposed to manage food limitation better), then this type of phenotypic matching could also account for the differences in plasticity that we observed. In this case, EPO might be

more resilient because they have been reared in an environment less conducive to their developmental needs.

It is important to acknowledge that not every result we obtained is consistent with the hypothesis outlined above. For example, WPO tended to produce more offspring per breeding attempt when contemporary breeding temperatures were variable. Thus, in this instance, WPO were responsive to contemporary environmental conditions (higher individual plasticity), and they performed better under conditions that we would assume are adverse. With respect to the second point, we have evidence from a previous study (this thesis, Chapter 2) that more variable ambient temperatures during incubation are associated with increased probability of fledgling recruitment. If incubation temperature variability is in fact a positive determinant of offspring fitness, it may simply be the case that variability during the breeding season as a whole results in higher reproductive success. This idea is additionally supported by this study's finding that WPO produced more lifetime fledglings when they had been incubated in more variable temperatures.

It is also important to consider that, despite having measured multiple life history outcomes across a number of years and environmental contexts, our study is actually rather limited in scope. In examining female recruits, we have ignored males, as well as those females that survived to adulthood and dispersed outside of our study area. But male life history may be an important target of selection for female extra-pair behavior, especially as it relates to extra-pair siring success (e.g. Gerlach et al. 2012). And if EPO are truly better at managing and mitigating stress, then it stands to reason that they may also be better adapted to withstand the stress of long-distance dispersal and settlement in novel environments. Both males and long-distance dispersers would benefit from further study.

To our knowledge, this study is the first to explicitly demonstrate differences in developmental plasticity between EPO and WPO, and to link these differences in plasticity to variation in adult life history outcomes. We suggest that EPO and WPO may represent alternative reproductive strategies, with investment into developmentally flexible WPO being favored when environmental conditions are benign and investment into developmentally

inflexible EPO being favored when environmental conditions are poor. While we did not observe any environmental context in which EPO outperformed WPO, we suggest that such contexts may exist, and encourage further research aimed at determining whether this is the case. This hypothesis has the potential to reconcile seemingly disparate results, including why benefits of EPP are only sometimes found (Akçay and Roughgarden 2007), why WPO are sometimes demonstrated to be of higher overall fitness than EPO (Hsu et al. 2014), and why rates of EPP are higher in more unpredictable environments (Botero and Rubenstein 2012). Thus, it deserves further study.

APPENDIX

CHAPTER 1 SUPPLEMENTAL TABLES

TABLES S1-S10: Full model selection for each metric of nestling fitness. For each metric, the best-supported model is italicized.

SURVIVAL TO FLEDGING

Model	k	ΔAIC	l_i	w_i
Extra-pair Status + Disturbance	3	0	1	0.30
<i>Disturbance</i>	2	0.6	0.74	0.22
Extra-pair Status*Disturbance	4	1.5	0.47	0.14
Extra-pair Status	2	3.5	0.17	0.05
Extra-pair Status + Predator Treatment	3	3.5	0.17	0.05
Predator Treatment	2	3.7	0.16	0.05
Intercept	1	4.0	0.14	0.04
Extra-pair Status + Side of Site	3	4.3	0.12	0.04
Side of Site	2	4.6	0.10	0.03
Extra-pair Status*Predator Treatment	4	5.0	0.08	0.02
Extra-pair Status*Side of Site	4	5.4	0.07	0.02
Extra-pair Status + Predator Disturbance	3	5.5	0.06	0.02
Predator Disturbance	2	5.9	0.05	0.01
Extra-pair Status*Predator Disturbance	4	6.6	0.04	0.01

DAY 6 HEAD-BILL

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	0.60
Intercept	1	3.3	0.19	0.11
Side of Site	2	4.1	0.13	0.08
Predator Treatment	2	4.6	0.10	0.06
Extra-Pair Status	2	4.9	0.09	0.05
Extra-pair Status + Side of Site	3	5.6	0.06	0.04
Extra-Pair Status + Predator Treatment	3	6.2	0.05	0.03
Extra-pair Status*Side of Site	4	7.5	0.02	0.01
Predator Disturbance	2	8.8	0.01	0.01
Disturbance	2	9.3	0.01	0.01
Extra-Pair Status + Predator Disturbance	3	10.4	0.01	0.01
Extra-Pair Status + Disturbance	3	11.0	0.00	0.00
Extra-Pair Status*Predator Disturbance	4	12.4	0.00	0.00
Extra-Pair Status*Disturbance	4	16.2	0.00	0.00

DAY 6 WING LENGTH

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	0.86
Extra-pair Status + Predator Treatment	3	6.0	0.05	0.04
Extra-pair Status*Side of Site	4	6.9	0.03	0.03
Extra-pair Status + Side of Site	2	7.0	0.03	0.03
Predator Treatment	2	7.9	0.02	0.02
Extra-pair Status	2	8.9	0.01	0.01
Side of Site	2	9.1	0.01	0.01
Extra-pair Status + Predator Disturbance	3	10.5	0.01	0.01
Intercept	1	10.9	0.00	0.00
Extra-pair Status*Predator Disturbance	4	12.0	0.00	0.00
Predator Disturbance	2	12.6	0.00	0.00
Extra-pair Status + Disturbance	3	12.9	0.00	0.00
Disturbance	2	14.8	0.00	0.00
Extra-pair Status + Disturbance	3	14.8	0.00	0.00

DAY 6 MASS

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	0.98
Predator Treatment	2	10.3	0.01	0.01
Side of Site	2	10.4	0.01	0.01
Intercept	1	11.5	0.00	0.00
Extra-pair Status + Predator Treatment	3	12.1	0.00	0.00
Extra-pair Status + Side of Site	3	12.2	0.00	0.00
Extra-pair Status*Side of Site	4	12.6	0.00	0.00
Extra-pair Status	2	13.2	0.00	0.00
Extra-pair Status*Predator Disturbance	4	13.4	0.00	0.00
Predator Disturbance	2	14.1	0.00	0.00
Extra-pair Status + Predator Disturbance	3	15.8	0.00	0.00
Disturbance	2	16.0	0.00	0.00
Extra-pair Status + Disturbance	3	17.8	0.00	0.00
Extra-pair Status*Disturbance	4	19.6	0.00	0.00

DAY 12 HEAD-BILL

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	0.88
Intercept	1	5.3	0.07	0.06
Side of Site	2	7.1	0.03	0.03
Predator Treatment	2	7.7	0.02	0.02
Extra-pair Status	2	9.0	0.01	0.01
Disturbance	2	10.4	0.01	0.01
Extra-pair Status*Predator Disturbance	4	10.8	0.00	0.00
Extra-pair Status + Side of Site	3	10.8	0.00	0.00
Extra-pair Status + Predator Treatment	3	11.4	0.00	0.00
Predator Disturbance	2	11.9	0.00	0.00
Extra-pair Status*Side of Site	4	13.0	0.00	0.00
Extra-pair Status + Disturbance	3	14.1	0.00	0.00
Extra-pair Status + Predator Disturbance	3	15.6	0.00	0.00
Extra-pair Status*Disturbance	4	19.9	0.00	0.00

DAY 12 WING LENGTH

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	0.99
Extra-pair Status*Side of Site	4	9.6	0.01	0.01
Side of Site	2	11.5	0.00	0.00
Extra-pair Status + Side of Site	3	11.6	0.00	0.00
Predator Treatment	2	12.6	0.00	0.00
Extra-pair Status + Predator Treatment	3	12.8	0.00	0.00
Extra-pair Status*Predator Disturbance	4	13.4	0.00	0.00
Intercept	1	14.6	0.00	0.00
Extra-pair Status	2	14.7	0.00	0.00
Disturbance	2	16.2	0.00	0.00
Predator Disturbance	2	16.2	0.00	0.00
Extra-pair Status + Disturbance	3	16.3	0.00	0.00
Extra-pair Status + Predator Disturbance	3	16.3	0.00	0.00
Extra-pair Status*Disturbance	4	17.3	0.00	0.00

DAY 12 MASS

Model	k	ΔAIC	l_i	w_i
<i>Extra-pair Status*Predator Treatment</i>	4	0	1	1
Intercept	1	10.8	0.00	0.00
Side of Site	2	11.1	0.00	0.00
Predator Treatment	2	11.6	0.00	0.00
Extra-pair Status*Side of Site	4	11.8	0.00	0.00
Extra-pair Status*Predator Disturbance	4	11.9	0.00	0.00
Extra-pair Status	2	13.1	0.00	0.00
Extra-pair Status + Side of Site	3	13.5	0.00	0.00
Extra-pair Status + Predator Treatment	3	13.9	0.00	0.00
Disturbance	2	15.1	0.00	0.00
Predator Disturbance	2	15.6	0.00	0.00
Extra-pair Status + Disturbance	3	17.4	0.00	0.00
Extra-pair Status + Predator Disturbance	3	17.9	0.00	0.00
Extra-pair Status*Disturbance	4	20.3	0.00	0.00

BASELINE CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.49
Side of Site	2	2.6	0.27	0.13
Predator Treatment	2	2.7	0.26	0.13
Extra-pair Status	2	2.9	0.23	0.11
Extra-pair Status + Side of Site	3	5.4	0.07	0.03
Extra-pair Status + Predator Treatment	3	5.5	0.06	0.03
Disturbance	2	6.3	0.04	0.02
Predator Disturbance	2	6.5	0.04	0.02
Extra-pair Status*Predator Treatment	4	7.3	0.03	0.01
Extra-pair Status*Side of Site	4	7.3	0.03	0.01
Extra-pair Status + Disturbance	3	9.1	0.01	0.00
Extra-pair Status + Predator Disturbance	3	9.4	0.01	0.00
Extra-pair Status*Disturbance	4	14.8	0.00	0.00
Extra-pair Status*Predator Disturbance	4	15.4	0.00	0.00

STRESS-INDUCED CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.54
Side of Site	2	2.9	0.23	0.12
Predator Treatment	2	3.0	0.22	0.12
Extra-pair Status	2	3.6	0.17	0.09
Disturbance	2	5.1	0.08	0.04
Extra-pair Status + Predator Treatment	3	6.5	0.04	0.02
Extra-pair Status + Side of Site	3	6.5	0.04	0.02
Predator Disturbance	2	6.8	0.03	0.02
Extra-pair Status*Predator Treatment	4	7.7	0.02	0.01
Extra-pair Status + Disturbance	3	8.8	0.01	0.01
Extra-pair Status*Side of Site	4	8.8	0.01	0.01
Extra-pair Status + Predator Disturbance	3	10.4	0.01	0.01
Extra-pair Status*Disturbance	4	11.3	0.00	0.00
Extra-pair Status*Predator Disturbance	4	16.7	0.00	0.00

CORTICOSTERONE STRESS RESPONSE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.47
Extra-pair Status	2	2.6	0.27	0.13
Side of Site	2	2.8	0.25	0.12
Predator Treatment	2	2.8	0.25	0.12
Disturbance	2	5.2	0.07	0.03
Extra-pair Status + Predator Treatment	3	5.4	0.07	0.03
Extra-pair Status + Side of Site	3	5.4	0.07	0.03
Extra-pair Status*Predator Treatment	4	5.6	0.06	0.03
Extra-pair Status*Side of Site	4	7.0	0.03	0.01
Predator Disturbance	2	7.1	0.03	0.01
Extra-pair Status + Disturbance	3	7.5	0.02	0.01
Extra-pair Status + Predator Disturbance	3	9.7	0.01	0.00
Extra-pair Status*Disturbance	4	12.2	0.00	0.00
Extra-pair Status*Predator Disturbance	4	14.9	0.00	0.00

TABLES S11-S31: Full model selection for male traits. For each male trait, the best-supported model is italicized.

SOCIAL MALE AGE

Model	k	ΔAIC	l_i	w_i
<i>Side of Site</i>	2	0	1	0.68
Side of Site + Predator Treatment	3	2.0	0.37	0.25
Intercept	1	5.7	0.06	0.04
Predator Treatment	2	6.6	0.04	0.03

SOCIAL MALE HEAD-BILL

Model	k	ΔAIC	l_i	w_i
<i>Side of Site</i>	2	0	1	0.64
Side of Site + Predator Treatment	3	1.9	0.39	0.25
Intercept	1	4.9	0.09	0.06
Predator Treatment	2	5.0	0.08	0.05

SOCIAL MALE WING LENGTH

Model	k	ΔAIC	l_i	w_i
<i>Side of Site</i>	2	0	1	0.59
Side of Site + Predator Treatment	3	1.3	0.52	0.31
Predator Treatment	2	4.3	0.12	0.07
Intercept	1	5.9	0.05	0.03

SOCIAL MALE MASS

Model	k	ΔAIC	l_i	w_i
<i>Side of Site</i>	2	0	1	0.64
Side of Site + Predator Treatment	3	1.9	0.39	0.25
Intercept	1	4.6	0.10	0.06
Predator Treatment	2	5.1	0.08	0.05

SOCIAL MALE BASELINE CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.32
Side of Site	2	0.4	0.82	0.26
Side of Site + Predator Treatment	3	0.4	0.82	0.26
<i>Intercept</i>	1	1.5	0.47	0.15

SOCIAL MALE STRESS-INDUCED CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.53
Predator Treatment	2	2.0	0.37	0.20
Side of Site	2	2.0	0.37	0.20
Side of Site + Predator Treatment	3	4.0	0.14	0.07

SOCIAL MALE CORTICOSTERONE STRESS RESPONSE

Model	k	ΔAIC	l_i	w_i
Side of Site + Predator Treatment	3	0	1	0.35
<i>Side of Site</i>	2	0.4	0.82	0.28
Predator Treatment	3	0.4	0.82	0.28
Intercept	1	2.8	0.25	0.09

EXTRA-PAIR MALE AGE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.47
Predator Treatment	2	1.3	0.52	0.25
Side of Site	2	1.8	0.41	0.19
Side of Site + Predator Treatment	3	3.3	0.19	0.09

EXTRA-PAIR MALE HEAD-BILL

Model	k	ΔAIC	l_i	w_i
<i>Side of Site</i>	2	0	1	0.40
Side of Site + Predator Treatment	3	0.8	0.67	0.27
Predator Treatment	2	1.5	0.47	0.19
<i>Intercept</i>	1	2.2	0.33	0.13

EXTRA-PAIR MALE WING LENGTH

Model	k	ΔAIC	l_i	w_i
<i>Predator Treatment</i>	2	0	1	0.55
Side of Site + Predator Treatment	3	0.9	0.64	0.35
Side of Site	2	4.3	0.12	0.07
<i>Intercept</i>	1	5.4	0.07	0.04

EXTRA-PAIR MALE MASS

Model	k	ΔAIC	l_i	w_i
Side of Site + Predator Treatment	3	0	1	0.60
<i>Side of Site</i>	2	1.8	0.41	0.25
Predator Treatment	2	2.9	0.23	0.14
<i>Intercept</i>	1	7.1	0.03	0.02

EXTRA-PAIR MALE BASELINE CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.38
<i>Intercept</i>	1	1.1	0.58	0.22
Side of Site + Predator Treatment	3	1.2	0.55	0.21
Side of Site	2	1.4	0.50	0.19

EXTRA-PAIR MALE STRESS-INDUCED CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.42
Side of Site	2	0.6	0.74	0.31
Predator Treatment	2	2.0	0.37	0.15
Side of Site + Predator Treatment	3	2.5	0.29	0.12

EXTRA-PAIR MALE CORTICOSTERONE STRESS RESPONSE

Model	k	ΔAIC	l_i	w_i
Side of Site + Predator Treatment	3	0	1	0.45
Side of Site	2	0.6	0.74	0.33
Predator Treatment	2	2.1	0.35	0.16
Intercept	1	4.2	0.12	0.05

EXTRA-PAIR MALE - SOCIAL MALE AGE

Model	k	ΔAIC	l_i	w_i
Intercept	1	0	1	0.45
Side of Site	2	1.0	0.61	0.27
Predator Treatment	2	1.9	0.39	0.17
Side of Site + Predator Treatment	3	2.9	0.23	0.10

EXTRA-PAIR MALE - SOCIAL MALE HEAD-BILL

Model	k	ΔAIC	l_i	w_i
Intercept	1	0	1	0.53
Side of Site	2	2.0	0.37	0.20
Predator Treatment	2	2.0	0.37	0.20
Side of Site + Predator Treatment	3	4.0	0.14	0.07

EXTRA-PAIR MALE - SOCIAL MALE WING LENGTH

Model	k	ΔAIC	l_i	w_i
Intercept	1	0	1	0.51
Predator Treatment	2	1.5	0.47	0.24
Side of Site	2	2.0	0.37	0.19
Side of Site + Predator Treatment	3	4.5	0.11	0.06

EXTRA-PAIR MALE - SOCIAL MALE MASS

Model	k	ΔAIC	l_i	w_i
Intercept	1	0	1	0.49
Predator Treatment	2	1.6	0.45	0.22
Side of Site	2	1.9	0.39	0.19
Side of Site + Predator Treatment	3	3.2	0.20	0.10

EXTRA-PAIR MALE - SOCIAL MALE BASELINE CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
Intercept	1	0	1	0.53
Side of Site	2	2.0	0.37	0.20
Predator Treatment	2	2.0	0.37	0.20
Side of Site + Predator Treatment	3	4.0	0.14	0.07

EXTRA-PAIR MALE - SOCIAL MALE STRESS-INDUCED CORTICOSTERONE

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.45
Side of Site	2	0.9	0.64	0.29
Predator Treatment	2	2.0	0.37	0.17
Side of Site + Predator Treatment	3	2.9	0.23	0.10

EXTRA-PAIR MALE - SOCIAL MALE CORTICOSTERONE STRESS RESPONSE

Model	k	ΔAIC	l_i	w_i
Side of Site	2	0	1	0.35
<i>Intercept</i>	1	0.2	0.90	0.31
Predator Treatment	2	1.3	0.52	0.18
Side of Site + Predator Treatment	3	1.6	0.45	0.16

TABLES S32-S42: Full model selection for female traits. For each female trait, the best-supported model is italicized.

SOCIAL FEMALE AGE

Model	k	ΔAIC	l_i	w_i
Side of Site	2	0	1	0.38
<i>Intercept</i>	1	0.3	0.86	0.33
Side of Site + Predator Treatment	3	1.7	0.43	0.16
Predator Treatment	2	2.2	0.33	0.13

SOCIAL FEMALE HEAD-BILL (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.38
<i>Intercept</i>	1	0.2	0.90	0.34
Side of Site	2	1.9	0.39	0.15
Side of Site + Predator Treatment	3	2.0	0.37	0.14

SOCIAL FEMALE WING LENGTH (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.51
Predator Treatment	2	1.8	0.41	0.21
Side of Site	2	1.9	0.39	0.20
Side of Site + Predator Treatment	3	3.5	0.17	0.09

SOCIAL FEMALE MASS (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.52
Predator Treatment	2	1.8	0.41	0.21
Side of Site	2	2.0	0.37	0.19
Side of Site + Predator Treatment	3	3.7	0.16	0.08

SOCIAL FEMALE MASS (NESTLING CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.52
Predator Treatment	2	1.8	0.41	0.21
Side of Site	2	2.0	0.37	0.19
Side of Site + Predator Treatment	3	3.8	0.15	0.08

SOCIAL FEMALE BASELINE CORTICOSTERONE (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.36
Predator Treatment	2	0.2	0.90	0.32
Side of Site + Predator Treatment	3	1.4	0.50	0.18
Side of Site	2	1.9	0.39	0.14

SOCIAL FEMALE STRESS-INDUCED CORTICOSTERONE (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.46
Side of Site	2	1.5	0.47	0.21
Predator Treatment	2	1.7	0.43	0.20
Side of Site + Predator Treatment	3	2.5	0.29	0.13

SOCIAL FEMALE CORTICOSTERONE STRESS RESPONSE (INCUBATION CAPTURE)

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.33
<i>Intercept</i>	1	0.3	0.86	0.28
Side of Site + Predator Treatment	3	0.3	0.86	0.28
Side of Site	2	2.1	0.35	0.11

SOCIAL FEMALE BASELINE CORTICOSTERONE (NESTLING CAPTURE)

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.31
Side of Site	2	0	1	0.31
Side of Site + Predator Treatment	3	0.7	0.70	0.22
<i>Intercept</i>	1	1.3	0.52	0.16

SOCIAL FEMALE STRESS-INDUCED CORTICOSTERONE (NESTLING CAPTURE)

Model	k	ΔAIC	l_i	w_i
<i>Intercept</i>	1	0	1	0.45
Side of Site	2	1.3	0.52	0.24
Predator Treatment	2	1.5	0.47	0.21
Side of Site + Predator Treatment	3	3.1	0.21	0.10

SOCIAL FEMALE CORTICOSTERONE STRESS RESPONSE (NESTLING CAPTURE)

Model	k	ΔAIC	l_i	w_i
Predator Treatment	2	0	1	0.39
Side of Site	2	1.1	0.58	0.23
Side of Site + Predator Treatment	3	1.1	0.58	0.23
<i>Intercept</i>	1	1.9	0.39	0.15

TABLE S43: Full model selection for nest visitation rate. The best-supported model is italicized.

NEST VISITATION RATE

Model	k	ΔAIC	l_i	w_i
<i>Nestling Age</i>	2	0	1	0.25
Brood Size + Nestling Age	3	0	1	0.25
Predator Treatment + Nestling Age	3	1.9	0.39	0.10
Side of Site + Nestling Age	3	2.0	0.37	0.09
Side of Site + Brood Size + Nestling Age	4	2.0	0.37	0.09
Predator Treatment + Brood Size + Nestling Age	4	2.0	0.37	0.09
Brood Size	2	4.0	0.14	0.03
Intercept	1	4.3	0.12	0.03
Predator Treatment + Brood Size	3	4.3	0.12	0.03
Predator Treatment	2	4.4	0.11	0.03
Side of Site + Brood Size	3	6.0	0.05	0.01
Side of Site	2	6.3	0.04	0.01

BIBLIOGRAPHY

- Aguillon, S. M. and R. A. Duckworth. 2015. Kin aggression and resource availability influence phenotype-dependent dispersal in a passerine bird. *Behavioral Ecology and Sociobiology* 69:625-633.
- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. 2nd International Symposium on Information Theory. (eds. B. N. Petrov and F. Csaki), 267-281. Akademiai Kiado, Budapest.
- Akçay, E. and J. Roughgarden. 2007. Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research* 9:855-868.
- Anderson, D. J. 1990. Evolution of obligate siblicide in boobies. 1. A test of the insurance-egg hypothesis. *American Naturalist* 135:334-350.
- Angelier, F., R. L. Holberton, and P. P. Marra. 2009. Does stress response predict return rate in a migratory bird species? A study of American redstarts and their non-breeding habitat. *Proceedings of the Royal Society of London B: Biological Sciences* 276:3545-3551.
- Arct, A., S. M. Drobniak, and M. Cichoń. 2015. Genetic similarity between mates predicts extra-pair paternity – a meta-analysis of bird studies. *Behavioral Ecology* 26:959-968.
- Arct, A., S. M. Drobniak, E. Podmokla, L. Gustafson, M. Cichoń. 2013. Benefits of extra-pair mating may depend on environmental conditions – an experimental study in the blue tit (*Cyanistes caeruleus*). *Behavioral Ecology and Sociobiology* 67:1809-1815.
- Arnqvist, G. and M. Kirkpatrick. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. *American Naturalist* 165(S5):S26-S37.
- Arnqvist, G. and L. Rowe, 2005, *Sexual conflict*. Princeton University Press. Princeton and Woodstock.
- Bentz, A. B., K. J. Navara, and L. Siefferman. 2013. Phenotypic plasticity in response to breeding density in Tree Swallows: an adaptive maternal effect? *Hormones and Behavior* 64:729-736.
- Blomqvist, D., M. Andersson, C. Küpper, I. C. Cuthill, J. Kis, R. B. Lanctot, B. K. Sandercock, T. Székely, J. Wallander, and B. Kempanaers. 2002. Genetic similarity between mates and extra-pair parentage in three species of shorebirds. *Nature* 419:613-615.
- Bonduriansky, R. 2009. Reappraising sexual coevolution and the sex roles. *PLoS Biology* 7(12):e1000255.

- Botero, C. A. and D. R. Rubenstein. 2012. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. *PLoS ONE* 7(2):e32311
- Bouwman, K. M. 2005. *The illusion of monogamy: patterns of extra-pair paternity in the Reed Bunting (Emberiza schoeniclus)*. (Doctoral Dissertation) University of Groningen. ISBN 90-367-2236-5.
- Bowers, E. K., A. M. Forsman, B. S. Masters, B. G. P. Johnson, L. S. Johnson, S. K. Sakaluk, and C. F. Thompson. 2015. Increased extra-pair paternity in broods of aging males and enhanced recruitment of extra-pair young in a migratory bird. *Evolution* 69:2533-2541.
- Branch, C. L., D. Y. Kozlovsky, and V. V. Pravosudov. 2015. Elevation-related differences in female mate preference in Mountain Chickadees: are smart chickadees choosier? *Animal Behaviour* 99:89-94.
- Breuner, C. W., J. C. Wingfield, and L. M. Romero. 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's White-crowned Sparrow. *Journal of Experimental Zoology* 284:334-342.
- Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320:800-803.
- Clutton-Brock, T. 2009. Sexual selection in females. *Animal Behaviour* 77:3-11.
- Constantini, D., N. B. Metcalfe, and P. Monaghan. 2010. Ecological processes in a hermetic framework. *Ecology Letters* 13:1435-1447.
- Cote, J. and J. Clobert. 2007. Social personalities influence dispersal in a lizard. *Proceedings of the Royal Society of London B: Biological Sciences* 274:383-390.
- DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution* 13:77-81.
- Dickens, M. J. and L. M. Romero. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. *General and Comparative Endocrinology* 191:177-189.
- Double, M. and A. Cockburn. 2000. Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. *Proceedings of the Royal Society of London B: Biological Sciences* 267:465-470.
- Duckworth, R. A. 2008. Adaptive dispersal strategies and the dynamics of a range expansion. *American Naturalist* 172:S4-S17.

- Duckworth, R. A., V. Belloni, and S. R. Anderson. 2015. Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science* 347:875-877.
- Dunn, P. O., R. J. Robertson, D. Michaud-Freeman, and P. T. Boag. 1994. Extra-pair paternity in Tree Swallows: why do females mate with more than one male? *Behavioral Ecology and Sociobiology* 35:273-281.
- Dunn, P. O. and L. A. Whittingham. 2007. Search costs influence the spatial distribution, but not the level, of extra-pair mating in Tree Swallows. *Behavioral Ecology and Sociobiology* 61:449-454.
- Edward, D. A. and T. Chapman. 2011. The evolution and significance of male mate choice. *Trends in Ecology and Evolution* 26:647-654.
- Ferretti, V. 2010. Variation in extra-pair mating systems in *Tachycineta* swallows: a life-history approach. PhD Dissertation. Cornell University.
- Forbes, S. 2014. Partial fertility and polyandry: a benefit of multiple mating hiding in plain sight? *Behavioral Ecology and Sociobiology* 68:1329-1334.
- Forsman, A. M., L. A. Vogel, S. K. Sakaluk, B. G. Johnson, B. S. Masters, L. S. Johnson, and C. F. Thompson. 2008. Female House Wrens (*Troglodytes aedon*) increase the size, but not immunocompetence, of their offspring through extra-pair mating. *Molecular Ecology* 17:3697-3706.
- Forstmeier, W., K. Martin, E. Bolund, H. Schielzeth, and B. Kempanaers. 2011. Female extrapair mating behavior can evolve via indirect selection on males. *Proceedings of the National Academy of Sciences USA* 108:10608-10613.
- Forstmeier, W., S. Nakagawa, S. C. Griffith, and B. Kempanaers. 2014. Female extra-pair mating: adaptation or genetic constraint? *Trends in Ecology and Evolution* 29:456-464.
- Garvin, J. C., B. Abroe, M. C. Pedersen, P. O. Dunn, and L. A. Whittingham. 2006. Immune response of nestling warblers varies with extra-pair paternity and temperature. *Molecular Ecology* 15:3833-3840.
- Gerlach, N. M., J. W. McGlothlin, P. G. Parker, and E. D. Ketterson. 2012. Promiscuous mating produces offspring with higher lifetime fitness. *Proceedings of the Royal Society of London B: Biological Sciences* 279:860-866.
- Gissing, G. J., T. J. Crease, and A. L. A. Middleton. 1998. Extrapair paternity associated with reneating in the American Goldfinch. *Auk* 115:230-234.
- Gray, E. M. 1997. Female Red-winged Blackbirds accrue material benefits from copulating with extra-pair males. *Animal Behaviour* 53:625-639.

- Griffith, S. C. 2007. The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. *American Naturalist* 169:274-281.
- Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11:2195-2212.
- Griffiths, R., S. Daan, and C. Dijkstra. 1996. Sex identification in birds using two CHD genes. *Proceedings of the Royal Society of London B:Biological Sciences* 263:1251-1256.
- Hansson, B., S. Bensch, and D. Hasselquist. 2004. Lifetime fitness of short- and long-distance dispersing Great Reed Warblers. *Evolution* 58:2546-2557.
- Heinig, A., S. Pant, J. Dunning, A. Bass, Z. Coburn, and J. F. Prather. 2014. Male mate preferences in mutual mate choice: finches modulate their songs across and within male-female interactions. *Animal Behaviour* 97:1-12.
- Herre, E. A. 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature* 329:627-629.
- Hess, P. J., C. G. Zenger, and R. A. Schmidt. 2008. Weather-related Tree Swallow mortality and reduced nesting effort. *Northeastern Naturalist* 15:630-631.
- Hill, C. E., C. Akçay, S. E. Campbell, and M. D. Beecher. 2011. Extrapair paternity, song, and genetic quality in song sparrows. *Behavioral Ecology* 22:73-81.
- Hoi, H., J. Krištofik, and A. Darolová. 2013. Experimentally simulating paternity uncertainty: immediate and long-term responses of male and female reed warblers *Acrocephalus scirpaceus*. *PLoS One* 8(4):e62541.
- Hoogland, J. L., K. E. Cannon, L. M. DeBarbieri, and T. G. Manno. 2006. Selective predation on Utah Prairie Dogs. *American Naturalist* 168:546-552.
- Hsu, Y.-H., J. Schroeder, I. Winney, T. Burke, and S. Nakagawa. 2014. Costly infidelity: low lifetime fitness of extra-pair offspring in a passerine bird. *Evolution* 68:2873-2884.
- Hsu, Y.-H., J. Schroeder, I. Winney, T. Burke, and S. Nakagawa. 2015. Are extra-pair males different from cuckolded males? A case study and a meta-analytic examination. *Molecular Ecology* 24:1558-1571.
- Jenkins, T. G., K. I. Aston, C. Pflueger, B. R. Cairns, and D. T. Carrell. 2014. Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. *PLoS Genetics* 10(7):e1004458
- Jennions, M. D. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society* 75:21-64.

- Jones, J. 2003. Tree Swallows (*Tachycineta bicolor*): a new model organism? *Auk* 120:591-599.
- Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16:1099-1106.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647-1649.
- Kempnaers, B. B. Congdon, P. Boag, and R. J. Robertson. 1999. Extrapair paternity and egg hatchability in Tree Swallows: evidence for the genetic compatibility hypothesis? *Behavioral Ecology* 10:304-311.
- Kempnaers, B., S. Everding, C. Bishop, P. Boag, and R. J. Robertson. 2001. Extra-pair paternity and the reproductive role of male floaters in the Tree Swallow (*Tachycineta bicolor*). *Behavioral Ecology and Sociobiology* 49:251-259.
- Kempnaers, B., G. R. Verheyen, M. Van den Broeck, T. Burke, C. Van Broeckhoven, and A. Dhondt. 1992. Extra-pair paternity results from female preference for high-quality males in the Blue Tit. *Nature* 357:494-496.
- Komdeur, J., S. Daan, J. Tinbergen, and C. Mateman. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. *Nature* 385:522-525.
- Kraft, P. G., C. E. Franklin, and M. W. Blows. 2006. Predator-induced phenotypic plasticity in tadpoles: extension or innovation? *Journal of Evolutionary Biology* 19:450-458.
- Krist, M., P. Nádvorník, L. Uvírová, and S. Bureš. 2005. Paternity covaries with laying and hatching order in the Collared Flycatcher *Ficedula albicollis*. *Behavioral Ecology and Sociobiology* 59:6-11.
- Lifjeld, J. T., P. O. Dunn, R. J. Robertson, and P. T. Boag. 1993. Extra-pair paternity in monogamous Tree Swallows. *Animal Behaviour* 45:213-229.
- Lifjeld, J. T. and R. J. Robertson. 1992. Female control of extra-pair fertilization in Tree Swallows. *Behavioral Ecology and Sociobiology* 31:89-96.
- Lima, S. L. 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. *Biological Reviews* 84:485-513.
- McCarty, J. P. 1995. Effects of short-term changes in environmental conditions on the foraging ecology and reproductive success of Tree Swallows, *Tachycineta bicolor*. PhD dissertation, Cornell University, Ithaca.

- McCarty, J. P. and D. W. Winkler. 1999. Relative importance of environmental variables in determining the growth of nestling Tree Swallows *Tachycineta bicolor*. *Ibis* 141:286-296.
- Makarewich, C. A., L. M. Stenzler, V. Ferretti, D. W. Winkler, and I. J. Lovette. 2009. Isolation and characterization of microsatellite markers from three species of swallows in the genus *Tachycineta*: *T. albilinea*, *T. bicolor*, and *T. leucorrhoa*. *Molecular Ecology Resources* 9:631-635.
- Marsh, R. L. 1980. Development of temperature regulation in nestling Tree Swallows. *Condor* 82:461-463.
- Mateo, J. M. 2007. Ecological and hormonal correlates of antipredator behavior in adult Belding's Ground Squirrels (*Spermophilus beldingi*). *Behavioral Ecology and Sociobiology* 62:37-49.
- Matsunami, M., J. Kitano, O. Kishida, H. Michimae, T. Miura, and K. Nishimura. 2015. Transcriptome analysis of predator- and prey-induced phenotypic plasticity in the Hokkaido salamander (*Hynobius retardatus*). *Molecular Ecology* 24:3064-3076.
- Moore, J. C., A. Loggenberg, and J. M. Greef. 2006. Kin competition promotes dispersal in a male pollinating fig wasp. *Biology Letters* 2:17-19.
- Morrow, E. H., Arnqvist, G., and T. E. Pitcher. 2002. The evolution of infertility: does hatching rate in birds coevolve with female polyandry? *Journal of Evolutionary Biology* 15:702-709.
- Neff, B. D. and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology* 14:19-38.
- O'Brien, E. L. and R. D. Dawson. 2007. Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine. *Behavioral Ecology and Sociobiology* 61:775-782.
- Pakanen, V.-M., O. Hildén, A. Rönkä, E. J. Belda, A. Luukkonen, L. Kvist, and K. Koivula. 2011. Breeding dispersal strategies following reproductive failure explain low apparent survival of immigrant Temminck's stints. *Oikos* 120:615-622.
- Palacios, M. G., J. E. Cunnick, D. W. Winkler, and C. M. Vleck. 2007. Immunosenescence in some but not all immune components in a free-living vertebrate, the Tree Swallows. *Proceedings of the Royal Society B: Biological Sciences* 274:951-957.
- Patrick, S. C., J. R. Chapman, H. L. Dugdale, J. L. Quinn, and B. C. Sheldon. 2012. Promiscuity, paternity, and personality in the great tit. *Proceedings of the Royal Society of London B: Biological Sciences* 279:1724-1730.

- Podmokla, E., A. Dubiec, A. Arct, S. M. Drobniak, L. Gustafsson, and M. Cichoń. 2015. Malaria infection status predicts extra-pair paternity in the Blue Tit. *Journal of Avian Biology* 46:303-306.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Richardson, D. S., J. Komdeur, T. Burke, and T. von Schantz. 2005. MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of London B: Biological Sciences* 272:759-767.
- Rodgers, A. B., C. P. Morgan, N. A. Leu, and T. L. Bale. 2015. Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. *Proceedings of the National Academy of Sciences* 112:13699-13704.
- Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21:55-89.
- Sardell, R. J., P. Arcese, L. F. Keller, and J. M. Reid. 2011. Sex-specific differential survival of extra-pair and within-pair offspring in song sparrows, *Melospiza melodia*. *Proceedings of the Royal Society of London B: Biological Sciences* 278:3251-3259.
- Schaub, M. and J. von Hirschheydt. 2009. Effect of current reproduction on apparent survival, breeding dispersal and future reproduction in barn swallows assessed by multistate capture-recapture models. *Journal of Animal Ecology* 78:625-635.
- Schmoll, T. 2011. A review and perspective on context-dependent genetic effects of extra-pair mating in birds. *Journal of Ornithology* 152(S1):265-277.
- Schmoll, T., V. Dietrich, W. Winkel, J. T. Epplen, F. Schurr, and T. Lubjuhn. 2005. Paternal genetic effects on offspring fitness are context dependent within the extrapair mating system of a socially monogamous passerine. *Evolution* 59:645-657.
- Schmoll, T., F. M. Schurr, W. Winkel, J. T. Epplen, and T. Lubjuhn. 2009. Lifespan, lifetime reproductive performance and paternity loss of within-pair and extra-pair offspring in the Coal Tit *Paripatus ater*. *Proceedings of the Royal Society of London B: Biological Sciences* 276:337-345.
- Schmoll, T. and O. Kleven. 2016. Functional infertility in a wild passerine bird. *Ibis*. doi: 10.1111/ibi.12376
- Sheldon, B. C. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proceedings of the Royal Society of London B: Biological Sciences* 257:25-30.

- Sinervo, B. and C. M. Lively. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* 380:240-243.
- Slagsvold, T. and J. T. Lifjeld. 1994. Polygyny in birds: the role of competition between females for male parental care. *American Naturalist* 143:59-94.
- Spitze, K. 1992. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. *American Naturalist* 139:229-247.
- Stapleton, M. K., O. Kleven, J. T. Lifjeld, and R. J. Robertson. 2007. Female Tree Swallows (*Tachycineta bicolor*) increase offspring heterozygosity through extrapair mating. *Behavioral Ecology and Sociobiology* 61:1725-1733.
- Steinberg, D. S., J. B. Losos, T. W. Schoener, D. A. Spiller, J. J. Kolbe, and M. Leal. 2014. Predation-associated modulation of movement-based signals by a Bahamian lizard. *Proceedings of the National Academy of Sciences* 111:9187-9192.
- Swaddle, J. P. and R. Lockwood. 2003. Wingtip shape and flight performance in the European Starling *Sturnus vulgaris*. *Ibis* 3:457-464.
- Taff, C. C. and C. R. Freeman-Gallant. 2016. Experimental tests of the function and flexibility of song consistency in a wild bird. *Ethology* 122:319-328.
- Tilgar, V., K. Moks, and P. Saag. 2011. Predator-induced stress changes parental feeding behavior in Pied Flycatchers. *Behavioral Ecology* 22:23-28.
- Townsend, A. K., A. B. Clark, and K. J. McGowan. 2010. Direct benefits and genetic costs of extrapair paternity for female American Crows (*Corvus brachyrhynchos*). *American Naturalist* 175:E1-E9.
- Vedder, O., M. J. L. Magrath, M. van der Velde, and J. Komdeur. 2013. Covariance of paternity and sex with laying order explains male bias in extra-pair offspring in a wild bird population. *Biology Letters* 9:20130616.
- Veen, T., T. Borge, S. C. Griffith, G-P. Sætre, S. Bures, L. Gustafsson, and B. C. Sheldon. 2001. Hybridization and adaptive mate choice in flycatchers. *Nature* 411:45-50.
- Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution* 17:313-320.
- Westneat, D. F. and I. R. K. Stewart. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics* 34:365-396.
- Whitman, D. W. and A. A. Agrawal. 2009. What is phenotypic plasticity and why is it important? Pages 1-63 in: D. W. Whitman and T. N. Ananthakrishna (editors), *Phenotypic plasticity of insects: Mechanisms and consequences*. Science Publishers, Inc, Enfield, NH.

- Whittingham, L. A. and P. O. Dunn. 2000. Offspring sex ratios in Tree Swallows: females in better condition produce more sons. *Molecular Ecology* 9:1123-1129.
- Whittingham, L. A. and P. O. Dunn. 2001. Survival of extrapair and within-pair young in Tree Swallows. *Behavioral Ecology* 12:496-500.
- Whittingham, L. A., P. O. Dunn, and M. K. Stapleton. 2006. Repeatability of extra-pair mating in Tree Swallows. *Molecular Ecology* 15:841-849.
- Wildy, E. L., D. P. Chivers, and A. R. Blaustein. 1999. Shifts in life-history traits as a response to cannibalism in larval Long-toed Salamanders (*Ambystoma macrodactylum*). *Journal of Chemical Ecology* 25:2337-2346.
- Wingfield, J. C. and Romero, L. M. 2001 Adrenocortical responses to stress and their modulation in free-living vertebrates. In *Handbook of Physiology* (eds B. S. McEwen & H. M. Goodman), pp. 211–234. New York, NY: Oxford University Press.
- Wingfield, J. C., J. P. Smith, and D. S. Farner. 1982. Endocrine responses of White-crowned Sparrows to environmental stress. *Condor* 84:399-409.
- Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *American Zoologist* 38:191-206.
- Winkler, D. W., K. K. Hallinger, D. R. Ardia, R. J. Robertson, B. J. Stutchbury, and R. R. Cohen. 2011. Tree Swallow (*Tachycineta bicolor*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/011doi:10.2173/bna.11>.
- Winkler, D. W., M. K. Luo, and E. Rakhimberdiev. 2013. Temperature effects on food supply and chick mortality in tree swallows (*Tachycineta bicolor*). *Oecologia* 173:129-138.
- Winkler, D. W., P. H. Wrege, P. E. Allen, T. L. Kast, P. Senesac, M. F. Wasson, P. E. Llambías, V. Ferretti, and P. J. Sullivan. 2004. Breeding dispersal and philopatry in the Tree Swallow. *Condor* 106:768-776.
- Winkler, D. W., P. H. Wrege, P. E. Allen, T. L. Kast, P. Senesac, M. F. Wasson, and P. J. Sullivan. 2005. The natal dispersal of Tree Swallows in a continuous mainland environment. *Journal of Animal Ecology* 74:1080-1090.
- Yasui, Y. 1998. The ‘genetic benefits’ of female multiple mating reconsidered. *Trends in Ecology and Evolution* 13:246-250.
- Yasui, Y. 2001. Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. *Ecological Research* 16:605-616.

- Yoder, J. M., E. A. Marschall, and D. A. Swanson. 2004. The cost of dispersal: predation as a function of movement and site familiarity in Ruffed Grouse. *Behavioral Ecology* 15:469-476.
- Yuta, T. and I. Koizumi. 2015. Does nest predation risk affect the frequency of extra-pair paternity in a socially monogamous passerine? *Journal of Avian Biology*.
DOI: 10.1111/jav.00713
- Zeh, J. A. and D. W. Zeh. 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society of London B: Biological Sciences* 263:1711-1717.
- Zeh, J. A. and D. W. Zeh. 1997. The evolution of polyandry II: post-copulatory defenses against genetic incompatibility. *Proceedings of the Royal Society of London B: Biological Sciences* 264:69-75.