REPRODUCTIVE TRADE-OFFS AND ENVIRONMENTAL CONSTRAINTS IN A MIGRATORY SONGBIRD

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Behavioral plasticity may allow organisms to respond adaptively to environmental constraints that limit investment in competing reproductive behaviors. For example, food limitation should reduce foraging success and cause males to reallocate effort among the competing demands of parental care, mate guarding, and extra-pair mating. Using both ecological and physiological approaches, I investigate how environmental constraints influence these reproductive trade-offs in the Black-throated Blue Warbler (*Setophaga caerulescens*), an insectivorous, migratory songbird with high rates of extra-pair mating and bi-parental care. In chapter 1, I investigate the mechanism by which environmental factors influence sex-specific parental provisioning and offspring condition. The association between low temperatures and food availability was an important environmental constraint favoring greater parental effort and cooperation to fledge offspring in good condition, but at the expense of other components of reproductive effort and parental condition. In chapters 2 and 3, I present results from a food supplementation study comparing fed and control males in habitats with low and high food abundance. I examine how food availability and endocrine physiology interact to regulate reproductive effort (chapter 2), and then examine the fitness consequences of individual variation in reproductive effort (chapter 3). Results from these studies indicate that plasticity in mating and parental effort allow birds to respond to changes in resource conditions that limit reproduction, and they also suggest a role for food in mediating hormone-regulated reproductive effort. Reduced environmental constraints increased male genetic reproductive success in both low and high quality habitats, but patterns of reproductive investment differed across habitats by affecting male reproductive trade-offs or female extra-pair mate choice. In chapter 4, I investigate the influence of environmental factors and reproductive trade-offs on the opportunity for extra-pair mating. Males on food-limited territories were more likely to sire extra-pair young and sire young further away from their territories compared to males on food-abundant territories, suggesting that food availability influenced foraging movements. Reproductive trade-offs constrained the timing of male extra-pair mating to the period when the social mate was incubating eggs. Together, these results indicate the importance of environmental constraints in mediating reproductive trade-offs and structuring interactions among extra-pair mates.

BIOGRAPHICAL SKETCH

Sara Ann Kaiser was born in 1978 in Waterloo, Iowa, a town nestled in a landscape mosaic of feed corn and soybeans. Although there were limited outdoor opportunities, her dad was an artist and a keen observer of wildlife and her mom was her first teacher, both instilling in her a strong sense of curiosity of the natural world. Her twin sister, Michelle, and older brother, Matthew, also shared this fascination. The three of them could be found chasing fireflies in the front lawn, catching crawdads in the creek, or digging for fossils in the rocks surrounding their home. As she grew older and her mom satisfied her requests to visit the St. Louis Zoo during trips to her grandparents' home, Sara found herself glued to the windows of the primate exhibits scribbling behavioral notes and sketching in her field book. On one memorable visit as a freshman in high school, she watched as a college-aged girl sat crouched in front of one of the windows making her own observations of a defiant gorilla. After a shy inquiry, she discovered that the girl was conducting a study for her ethology class. From that day on, Sara pronounced to her classmates and teachers that she was going to become an ethologist and study animal behavior.

As a sophomore at Iowa State University, Sara was preparing to attend Kenyatta University outside of Nairobi, Kenya, a program she sought out because it involved human-environment issues of African savanna cultivation and management policies of game reserves. Unfortunately, the program was abruptly cancelled, which resulted in her last-minute enrollment in an ornithology course at Iowa Lakeside Laboratory located in the Iowa Great Lakes region. Initially this was not her first choice, but the enthusiasm for birds from her instructor, Neil Bernstein, was instantly contagious and she became fascinated by the diversity of birds and their songs, and the challenge of their identification. The course provided her first objective exposure to conservation issues affecting bird populations in the Midwest. In the fall, she returned to Iowa State, shifted her research interests to ornithology, and joined Carol Vleck's avian physiology lab. In Vleck's lab, she studied the physiology of bird migration, found a second home, learned what it meant to be a part of a lab group, and discovered the bird community at her first ornithological conference. Sara graduated with distinction with a B.S. in Zoology and Genetics.

Over a decade since that field ornithology course, Sara has had extensive experience in teaching, field and laboratory research, and conservation management in the behavioral ecology, endocrinology, and population ecology of birds. Sara earned her M.S. from Michigan State University in 2004 from the Ecology, Evolutionary Biology and Behavior Program working with Catherine Lindell. She focused on one of the mechanisms through which the fitness of forest-interior birds may be affected by forest fragmentation. In addition to research, Sara has sought opportunities to improve her teaching outside of the traditional classroom setting. After obtaining her M.S., she worked for International Student Volunteers teaching natural resource management to undergraduates in New Zealand through field-based conservation projects. Prior to enrolling back in graduate school, Sara worked as a project manager for a non-profit wildlife organization, the Institute for Wildlife Studies, managing a long-term demographic study of a threatened bird endemic to the California Channel Islands. While she worked full-time as a project manager, Sara also initiated a citizen-science bird monitoring and banding study with PRBO Conservation Science at Cabrillo National Monument. These projects provided her unique opportunities to engage public interest and taught her how to convey science and research to broad audiences. Most importantly, these experiences solidified her professional pursuit of positions that integrate conservation science, education/training, and public awareness. In 2008, Sara returned to graduate school to pursue her Ph.D. and continue her research and education.

For my family

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Neurobiology and Behavior (NBB), Ecology and Evolutionary Biology, and Statistical Science. Thanks to the administrative staff in NBB and Macaulay Library for making my life as a graduate student easier. In particular, I thank Terri Natoli and Nancy Schrempf. My labwork was launched by extremely helpful discussions with Ned Place and Laura Stenzler. With their help, I never felt like I was stuck for very long without a solution. My transition from Washington to New York would not have been the same without my friends at the Lab of Ornithology. I would like to especially thank those that regularly attended “Ladies night”: Yula Kapetanakos, Scott Taylor, Becky Cramer, Amanda Talaba, Nancy Chen, Anna Forsman, Becca Brunner, Petra Deane, and Kristin Hook. I am so lucky to have such amazing women in my life. I am especially grateful to Benjamin Risk for brightening the last two years of this experience, being patient during my endless discourse about Black-throated Blue Warblers, and providing valuable assistance. Lastly, I want to thank my loving family for always supporting my endeavors. My parents, Cynthia and David, embraced my free spirit and provided me the freedom to explore my own interests. My brother, Matthew, and sister-in-law, Angie, always showed interest in my research and reminded me of the importance of finding balance in life and spending your time doing what you enjoy. Most importantly, I am indebted to my twin sister, Michelle, for her unconditional love and friendship since the day we both opened our eyes. Through the highs and the lows, she has always been there for me. I can honestly say that I would not have earned this achievement without her support and encouragement.

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PREFACE

Migratory birds often face variable and unpredictable environmental conditions upon arrival at their breeding grounds. The mechanism that enables individuals to adjust their reproductive effort to respond to variable environmental conditions is vital to their survival, reproductive success, and population stability. Behavioral plasticity may allow animals to respond adaptively to environmental conditions that limit investment in competing reproductive behaviors and shape life history strategies. Plasticity in reproductive behaviors in response to resource conditions is advantageous when it allows individuals to achieve high fitness across a broad range of environmental conditions. This plasticity depends on the hormonal mechanisms regulating the expression of reproductive behaviors and the environmental cues animals use to regulate reproduction. My dissertation focuses on how behavioral and hormonal plasticity may allow birds to respond to environmental factors that influence reproductive trade-offs, the hormonal mechanisms mediating reproductive trade-offs, and the fitness consequences of individual reproductive deicisons in the Black-throated Blue Warbler (*Setophaga caerulescens*), a Nearctic-Neotropical migratory songbird with high rates of extra-pair mating and bi-parental care.

In Chapter 1, I investigated the environmental factors that promote or constrain parental care to understand how males and females are selected to optimize their parental effort to fledge offspring in good condition under particular environmental conditions. In species with bi-parental care, males and females can conflict over how much time and energy to allocate to parental effort at the expense of other components of reproductive effort or parental survival. Males and females might differ substantially in the optimal parental effort that maximizes fitness returns when individual costs are greater for one sex. The potential costs associated with parental care (*e.g.,* reduced body condition), however, may be greater for females if variability in environmental factors, such as weather and food availability, leads to reduced male parental care. I hypothesized that sexual conflict may be greatest when weather reduces food availability and increases thermoregulatory demands. To test this hypothesis, I used long-term behavioral data collected from 1995 – 2011 to investigate the potential mediating effects of temperature, rainfall, and food availability on male and female parental provisioning, and also how adjustments in parental provisioning in response to these environmental factors affected offspring condition. I found that food availability mediated the association between temperature and parental provisioning, such that both sexes reduced their rate of provisioning when food was more abundant. Male parental investment in each brood was more variable than female parental investment and was correlated with offspring condition. Consistently high female parental investment in each brood likely accounted for seasonal declines in female body condition. This study indicates that the association between low temperatures and food availability is an important environmental constraint favoring greater parental effort and cooperation to fledge offspring in good condition, but potentially at the expense of other components of reproductive effort and parental condition.

In Chapter 2, I used a food supplementation experiment to examine plasticity in behavioral and hormonal responses to food availability, and tested whether plasma hormones mediated reproductive trade-offs between mating and parental effort. Behavioral plasticity can allow organisms to adjust to changing environmental cues, such as food availability, that regulate reproduction. However, adaptive behavioral plasticity in response to changing resource conditions will depend on how food affects the underlying hormonal mechanisms regulating reproductive behaviors. In birds, there is support for the role of androgens in regulating male reproductive trade-offs between mating and parental effort. Recent correlative evidence also demonstrates a role for baseline corticosterone (CORT) in regulating parental effort. This study focused on the role of plasma androgens and CORT in regulating male reproductive trade-offs on food-supplemented territories versus control territories in habitats with low and high food abundance. I hypothesized that energetic constraints due to food limitation would affect 1) the relative allocation of male reproductive effort to mating and parental behaviors, 2) the hormones regulating male reproductive behaviors, and 3) female parental effort. Selection on adaptive hormone-mediated reproductive trade-offs in response to environmental variation should occur at the level of the individual. Therefore, the second aspect of the study examined individual variation in the relationships between plasma hormones and male reproductive behaviors. The effects of supplemental feeding were most pronounced in low quality habitat, where fed males appeared to increase their mate guarding effort, had lower androgens concentrations, and were in better body condition relative to control males. Fed males also benefited indirectly from access to supplemental food because fed females provided more parental effort than did control females. Fed males, however, had higher baseline CORT during the parental stage than control males, possibly because fed males invested greater effort in extra-pair mate attraction at that time. Mating effort was positively associated with androgen concentrations, but parental effort was not associated with androgen or baseline CORT concentrations. This study indicates that adjustments in mating and parental effort allow birds to respond to changes in resource conditions that constrain reproduction, and they suggest a role for food in mediating hormone-regulated reproductive effort. In addition, these results suggest that plasma androgen is not the primary mediator of reproductive trade-offs in the Black-throated Blue Warbler, nor does baseline CORT regulate parental effort.

In Chapter 3, I determined the fitness consequences of individual variation in reproductive behavioral investment by fed and control males in habitats with low and high food abundance. Environmental constraints are hypothesized to affect the fitness costs and benefits associated with investment in extra-pair mating. Environmental factors that contribute to variability in extra-pair paternity among individuals within populations could influence genetic reproductive success. This study was a follow-up to Chapter 2 and utilized the food supplementation experiment, as well as natural variation in per-territory food availability, to test the environmental constraints hypothesis. Food supplementation increased total annual genetic reproductive success of males in both low and high quality habitats, but specific patterns of male reproductive investment differed across habitats. Food supplementation and naturally high food availability resulted in high levels of within-pair paternity (WPP), regardless of habitat. However, food supplementation in low quality habitat resulted in low levels of extra-pair paternity (EPP) and increased the likelihood of double-brooding, whereas fed and control males did not differ in these fitness measures in high quality habitat. The results of this study provide support for the hypothesis that environmental constraints can strongly affect individual variation in extra-pair paternity by either influencing male reproductive trade-offs or female reproductive decisions. Males in low quality habitat with experimentally reduced energetic constraints appeared to invest additional resources into mate guarding over pursuing extra-pair copulations, which resulted in greater fitness returns. Habitat quality influenced opportunities for extra-pair mating and the likelihood of double-brooding, contributing to variability in total annual genetic reproductive success among males.

In Chapter 4, I examined the spatial and temporal variation in individual patterns of EPP to determine under what environmental and social contexts an individual is likely to invest in securing WPP over gaining EPP to maximize their total annual genetic reproductive success.

Environmental factors that structure the distribution of potential mates, such as food availability, might affect the probability of EPP. Extra-pair males with food-abundant territories might have a higher probability of siring extra-pair young (EPY) and be able to travel further distances to pursue extra-pair copulations (EPC) due to reduced energetic constraints. Males are also assumed to be temporally limited to seek extra-pair copulations because trade-offs exist between guarding social mates and pursuing EPC during the nest building and egg-laying nest stages (*i.e.* fertile period) and between pursuing EPC and providing parental care during the nestling and post-fledging nest stages (*i.e.,* parental period). I hypothesized that territory quality and reproductive trade-offs would influence where and when males sired EPY. Extra-pair mating opportunities were mostly, but not entirely, limited to neighbors' nests, and males reduced reproductive trade-offs primarily by siring EPY after their social mates began incubation but before their young hatched. Males with high quality territories had a lower probability of siring EPY than males with low quality territories, and also were less likely to sire EPY in nests far away from their territories. We detected no effect of local breeding density on the distance between females and their extra-pair mates. These results indicate that factors affecting the relative timing of breeding between a male's social mate and available fertile females may have a strong effect on the opportunity for extra-pair mating. Reproductive trade-offs appear to constrain male extra-pair behavior in black-throated blue warblers, rather than spatial factors.

To summarize, extra-pair mating is typically discussed as being socially-mediated, but the physical environment structures the social environment. My dissertation research indicates the importance of the physical environment in mediating reproductive trade-offs involving mate guarding, pursuing EPC, and providing parental care, which affected total annual genetic reproductive success of Black-throated Blue Warbler males. Males that settle in high quality (*i.e.,* food abundant) habitat had high total annual genetic reproductive success because of a higher likelihood of their social mates producing two broods containing young that were genetically their own, and also siring some EPY. Males that settle in low quality (*i.e,* food-limited) habitat had low total annual genetic reproductive success. These males gained more EPP, but this reproductive investment had the lowest fitness return when EPP was lost and their social mates had a reduced likelihood of double-brooding.

To conclude, experimentally reducing food limitation for birds breeding in low quality habitat increased reproductive effort for both males and females. Reproductive behaviors thus showed a plastic response to resource conditions and the underlying hormonal mechanisms regulating behavioral expression were also responsive to changes in resource conditions. These results suggest that predicted shifts in Black-throated Blue Warbler habitat quality due to climate change, could be mitigated by behavioral plasticity in mating and parental effort.

CHAPTER 1

ENVIRONMENTAL FACTORS THAT AFFECT RESOURCE AVAILABILITY CONSTRAIN PARENTAL EFFORT AND REDUCE SEXUAL CONFLICT IN A MIGRATORY SONGBIRD*

*** S. A. Kaiser, T. S. Sillett, B. B. Risk, N. L. Rodenhouse, R. T. Holmes, and M. S. Webster**

ABSTRACT. In species with biparental care, males and females can conflict over how much time and energy to allocate to parental effort at the expense of other components of reproductive effort or parental survival. Males and females might differ substantially in the optimal parental effort that maximizes fitness returns when individual costs are greater for one sex. Environmental factors that affect resource availability and constrain parental effort may mediate sexual conflict over parental care and affect offspring condition. We hypothesized that sexual conflict may be greatest when weather reduces food availability and increases thermoregulatory demands. We used path analysis to elucidate the mechanism by which environmental factors influence sex-specific parental provisioning and offspring condition in a migratory songbird with bi-parental care, the Black-throated Blue Warbler (*Setophaga caerulescens*), at the Hubbard Brook Experimental Forest, New Hampshire, USA, 1995-2011. We found that: 1) food availability mediated the association between temperature and parental investment, 2) individual variation in male parental investment was correlated with offspring condition, 3) both sexes responded similarly to environmental variability, and 4) female parental investment in each brood was greater and less variable than male parental investment, likely accounting for the observed seasonal declines in female body condition. Our study indicates that the association between low temperatures and food availability is an important environmental constraint favoring greater parental effort and cooperation to fledge offspring in better condition, but potentially at the expense of other components of reproductive effort and parental condition. We suggest that sexual conflict may increase as the breeding season progresses because of greater accumulated reproductive costs to females resulting in reduced reproductive value of offspring.

INTRODUCTION

In species with biparental care, conflict can arise over parental investment, leading to a reduction in provisioning by one parent at the expense of the other (i.e., sexual conflict; Trivers, 1972) or of the quality of their offspring (i.e., parent-offspring conflict; Trivers, 1974), both of which have fitness consequences (Royle et al. 2002). Parental provisioning is essential for successful reproduction in many species (e.g., 80% of bird species, Cockburn, 2006), but involves significant energetic costs and constrains competing fitness-related behaviors (Clutton-Brock 1991). The energy a parent expends foraging and delivering food to its offspring might reduce its own body condition, which can decrease survival and the probability of future reproduction (Owens and Bennett 1994, Liker and Székely 2005). For males, the temporal constraints of provisioning young might also diminish their opportunity to gain extra-pair matings (reviewed in Magrath and Komdeur 2003) or to invest in mate guarding and territorial defense (Wingfield et al. 1990, Ketterson et al. 1992, Pryke and Griffith 2009). Thus, individuals must decide how to allocate their limited time and effort to parental care to maximize fitness returns for their effort (Westneat et al. 1990, Magrath and Komdeur 2003). For both males and females, fitness gains may differ substantially between low and high parental investment strategies depending on particular environmental factors (Magrath and Komdeur 2003). An examination of the environmental factors that promote or constrain male and female parental care under a range of environmental conditions will provide insight into what factors mediate sexual conflict over parental care.

Male investment in parental care generally exhibits greater variance among individuals than does investment among females (Olson et al. 2008). Experimental manipulations of parental effort show that females may partially compensate for reduced mate participation in feeding more than males do (reviewed in Harrison et al. 2009), and that total parental effort is most closely associated with male effort (Olson et al. 2008). Accordingly, ecological pressures selecting for greater parental effort should lead to increased male effort, whereas selection on males for reduced parental effort would be expected to lead to lower total parental care (Olson et al. 2008), which could reduce offspring quality.

Environmental conditions might influence the partitioning of provisioning behavior between males and females (Olson et al. 2008, Harrison et al. 2009, Westneat et al. 2011). For example, the disparity between the sexes in parental investment may be greatest when temperature and rainfall impose constraints by increasing thermoregulatory demands of parents or offspring (Spencer and Bryant 2002, Weathers et al. 2002, Johnson et al. 2007). Environmental conditions might also limit the availability or acquisition of food (Wittenberger 1982, Dawson and Bortolotti 2000, Low et al. 2011), thereby affecting adult body condition (Naef-Daenzer and Keller 1999, Tremblay et al. 2005, Low et al. 2008). Alternatively, food limitation and adverse weather might favor greater participation in provisioning by both parents to offset increased thermoregulatory demands of nestlings (Lyon et al. 1987, Dawson and Bortolotti 2000, Rauter et al. 2000, Hoset et al. 2005). Because female birds generally have higher mortality (Liker and Székely 2005), life history theory predicts that they should adjust their parental effort less than males in response to the effects of adverse weather on food resources and body condition because of sex differences in the sensitivity of mortality to parental effort, and that female parental investment in current broods should be greater than male investment (Roff 2002). Thus, optimal parental investment may differ between males and females depending, in part, on the mechanisms linking weather and provisioning behavior.

Behavioral responses to weather have been demonstrated in birds (e.g., Møller 2011), but the mechanisms by which weather influences sex-specific parental responses, and the consequences of those responses for offspring condition remain largely unstudied. For example, how does food availability or adult body condition mediate the effect of temperature and rainfall on parental effort and offspring condition? We also do not know whether the potential costs associated with parental care (*e.g.,* reduced body condition) are greater for females if variability in weather and food availability leads to reduced male parental care. An understanding of such ecological complexities is needed to understand how males and females are selected to optimize their parental effort under particular environmental conditions.

We investigated the potential mediating effects of temperature, rainfall, and food availability on male and female parental effort and how adjustments in parental effort in response to these environmental factors affected offspring condition. We used long-term data on parental provisioning by an insectivorous, migratory songbird with bi-parental care, the Black-throated Blue Warbler (*Setophaga caerulescens*). First, we hypothesized that temperature and rainfall would directly affect parental provisioning due to reduced foraging activity imposed by adverse weather (*Hypothesis 1A*), or that temperature and rainfall would be linked indirectly to parental provisioning through negative effects of weather on food availability or adult body condition (*Hypothesis 1B*). Hypotheses 1A and 1B are not mutually exclusive, allowing us to consider the relative influence of both direct and indirect effects of weather on parental provisioning. Second, we hypothesized that weather effects on parental provisioning would influence offspring condition (*Hypothesis 2*). Third, we hypothesized that females, which have lower annual apparent survival than males at our study site (Sillett and Holmes 2002), would respond less to environmental variability than males (*Hypothesis 3*). Lastly, we hypothesized that female parental investment in each brood would be greater than male parental investment, and that male parental investment would be more variable than that of females (*Hypothesis 4*).

METHODS

Study species and site

The Black-throated Blue Warbler is a sexually dichromatic, Nearctic-Neotropical migratory songbird that breeds in deciduous or mixed deciduous-coniferous forests in eastern North America (Holmes et al. 2005) and is insectivorous during the breeding season (Robinson and Holmes 1982, Holmes et al. 1986). Males aggressively defend territories and show strong site fidelity between years (Holmes et al. 2005). Females choose nest sites and build open cup nests in the shrub layer (Holmes et al. 2005). When conditions are favorable, pairs attempt second broods after successfully fledging first broods (Nagy and Holmes 2005). Females incubate eggs for 12 days, and both parents provision nestlings for *ca.* 9 days prior to their fledging. Females also brood nestlings during inclement weather (Holmes et al. 2005). Both sexes provision nestlings post-hatching and adjust their feeding rates to brood age and number of young (first brood: median = 4, second brood: median = 3), but provisioning rates are not significantly correlated with time of day (Goodbred and Holmes 1996). Nearly 90% of identifiable prey delivered to nestlings are Lepidoptera larvae (Rodenhouse and Holmes 1992, Goodbred and Holmes 1996).

Our analyses used data collected (from May-August), 1995 to 2011 as part of a long-term behavioral and demographic study of a population of *S. caerulescens* breeding in the Hubbard Brook Experimental Forest (HBEF), Woodstock, New Hampshire, USA (43°56'N, 71°45'W). We used data from three study plots established at low (250-350 m; 85 ha), mid (450-600 m; 65 ha), and high (750-850 m; 35 ha) elevations (Rodenhouse et al. 2003). The 3160 ha HBEF consists of unfragmented northern hardwood forest with a canopy dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*) that grades into boreal forest at higher elevations along the ridgelines (Schwarz et al. 2003). The understory is vegetated primarily by hobblebush (*Viburnum alnifolium*), the principal nest substrate for *S. caerulescens* in northern New England (Holmes et al. 2005), striped maple (*A. pensylvanicum*), and saplings of canopy species.

Warbler habitat quality and environmental conditions differed over the 600 m elevational gradient encompassed by our three study plots. On average, birds nesting at high elevations experienced cooler (approximately 2°C) and wetter (about 1.8 cm more rainfall) conditions than those nesting at low elevations. Biomass of Lepidoptera larvae was about two times greater at the high elevation plot, and the density of deciduous leaves in the understory, where warblers feed and nest, was approximately 50% greater compared to the low elevation plot, with intermediate values at the mid-elevation plot (Rodenhouse et al. unpubl. data). The abundance of nest predators, mainly red squirrels (*Tamiasciurus hudsonicus*), eastern chipmunks (*Tamias striatus*), and Blue Jays (*Cyanocitta cristata*), declined with elevation (Rodenhouse et al. unpubl. data).

Field methods

We monitored reproductive activity and demographic rates throughout each breeding season. Adults were captured with mist nets and marked individually with a unique combination of three plastic, color leg bands and one U.S. Geological Survey aluminum band. Males were typically lured to mist nets with song playback, and females were captured with mist nets near their nests. We measured the length of each warbler’s right tarsus to the nearest 0.01 mm and its mass to the nearest 0.1 g. We calculated size-corrected body mass from the residuals of a linear regression of mass on tarsus length (females: $F_{1,313} = 4.79$, $P = 0.03$, $R^2 = 0.02$; males: $F_{1,275} = 9.67$, $P < 0.01$, $R^2 = 0.03$) as an index of body condition. We restricted our analyses of body condition to adults captured within 14 days after their young hatched, to ensure that this index reflected the period of nestling provisioning. We weighed young on the sixth day (hatching = day 0) of their 9-day nestling stage, and used mean nestling mass per brood as an index of offspring body condition. We observed males defending territories and interacting with neighboring conspecifics to map territory boundaries relative to each plot's 50x50 m grid system. Mapping began when males arrived in early May and continued every 2-4 days until territories broke down in late July. Nests were found through intensive searching, and were monitored every other day throughout all nest stages with daily checks near anticipated hatch and fledge dates.

Parental provisioning. – We collected data on parental provisioning by videotaping nests for two hours between 0700 and 1100 on the seventh day of the nestling stage. We used camouflaged video cameras (*e.g.,* JVC Everio GZ-MG155U) mounted on a tripod at nest height but about 10 m distant from the nest. We transcribed videos starting 10 min after recording began to avoid any initial parental response to the presence of the video camera. During each adult visit to the nest, we confirmed the feeding parent's color bands and recorded its arrival time. We calculated the number of provisioning visits to the nest per hour ("provisioning rate") for males and females separately, and standardized for differences in brood size by dividing provisioning rate by brood size. Results did not differ when we assessed parental effort without standardizing by brood size (data not shown).

Index of caterpillar biomass per territory. – We estimated an index of caterpillar biomass available to provisioning *S. caerulescens* in each territory, based on visual caterpillar surveys (1997-2011) and kriged surfaces of understory leaf density derived from understory leaf sampling (1997-2001, 2007-2009) on our study plots; see Appendix 1.1 for details. Briefly, we counted and measured caterpillars on striped maple and hobblebush leaves in four biweekly surveys during the breeding season (1 June – 31 July), and then estimated leaf abundances for these two species on each territory. We calculated caterpillar biomass per leaf for each survey period and then multiplied this quantity by leaf abundances within warbler territories. For each nest, we used the index of caterpillar biomass from the survey period coinciding with the nestling stage. Our method accounted for inter-annual, seasonal, and spatial variability in caterpillar abundances on the two plant species most commonly used as foraging substrate by *S. caerulescens*.

Weather. – Ambient temperature (°C) and daily precipitation (mm) were measured hourly at three permanent, U.S. Forest Service weather stations, one adjacent to each study plot. To examine effects of temperature on caterpillar biomass, adult body condition, parental provisioning, and offspring body condition, we used the mean maximum daily temperature experienced during the nestling stage (from the hatch date through the day of videotaping; day 0–7) of each nest. Mean maximum daily temperature and adult body condition measured during the nestling stage were uncorrelated (females: $t_{76} = -0.6$, $P = 0.56$, $R^2 = 0.004$; males: $t_{79} = -1.1$, $P = 0.28$, $R^2 = 0.02$), but we detected a lag effect of mean maximum daily temperature on adult body condition (see Results). Therefore, we related adult body condition during the nestling stage to the mean maximum daily temperature experienced during the incubation stage (from the date the last egg was laid through the day prior to hatch). To investigate the effects of rainfall, we used total daily precipitation accumulated during the nestling stage of each nest. We did not detect a lag effect of rainfall during the incubation stage on adult body condition during the nestling stage.

Statistical analyses

Environmental variability. – We used univariate, general linear models to detect trends in temperature, rainfall, and caterpillar biomass both within seasons and across years at each elevation. We created three models with either mean maximum daily temperature, mean accumulated daily rainfall, or caterpillar biomass as response variables, and survey period (1-14 Jun, 15-30 Jun, 1-14 Jul, 15-31 Jul), elevation (low, mid, high), year, survey period × elevation, survey period × year, and elevation × year as fixed explanatory factors. In the rainfall model, we used a White-Huber corrected covariance matrix in the ANOVA to account for heteroscedasticity (White 1980). In the caterpillar model, we used the natural log of caterpillar biomass to improve normality of the residuals and we used the partial sums of squares in the ANOVA to account for unbalanced data (Fox 2008). We used path analyses (see below) to estimate the influence of temperature and rainfall on caterpillar biomass during the nestling stage for each individual nest. All analyses were conducted in R version 2.13.5 (R Core Team 2013).

Path model. – We used the R package "lavaan" (Rosseel 2012) to specify path models to test alternative hypotheses for how weather might influence parental provisioning and offspring condition for each sex (*Hypotheses 1A, 1B, 2*). Path analyses, in contrast to multivariate regression, can evaluate mediating terms (Y) between predictor (X) and response variables (Z) represented as a pathway ($X \rightarrow Y \rightarrow Z$), where Y directly affects Z and X indirectly affects Z (Pugesek et al. 2003). We designed candidate path models by altering the hypothesized effects of temperature and rainfall on caterpillar biomass, adult body condition, parental provisioning, and offspring condition and included the average elevation of each male's territory as a covariate in each path model. Elevation was estimated using 1-m resolution digital elevation models of the

HBEF generated from LiDAR (Light Detection and Ranging) data (Swatantran et al. 2012) using ArcGIS 10 (ESRI 2011). We were unable to explicitly examine inter-annual variability in parental provisioning in our path analysis because too few nests were videotaped in some years. Standardized path coefficients measuring the direct effects of the predictor variables on all response variables were estimated using full information maximum likelihood (Arbuckle 1996). The indirect effects and total effects (additive direct and indirect effects) were calculated in lavaan using the correlations and standardized direct effects among all variables. We used χ^2-statistics, the root mean square error (RMSE), and the comparative fit index (CFI) to test the goodness-of-fit of the models, where $P > 0.05$, RMSE < 0.05, and CFI > 0.95 indicated a well-supported model (Rosseel 2012). The full path model with all hypothesized paths is given in Appendix 1.1 (Figure S1). To assess sex differences in response to environmental conditions (*Hypothesis 3*), we tested whether the path coefficients in the female and male path models were equivalent. We calculated a *t*-statistic as the difference between coefficients in the male and female models divided by the square root of the sum of the variance of each coefficient.

Sex differences in parental provisioning. – We examined seasonal variability in female and male parental provisioning between first and second broods (*Hypothesis 4*). We defined second broods as nest attempts following a successfully fledged first brood; re-nesting attempts following a failed nest, but before any successful nest, were considered first broods. We used the Wilcoxon signed rank test to conduct paired-sample comparisons of: 1) differences in provisioning rate between broods by sex for 35 pairs with data from both first and second broods within the same season, 2) the difference between females and their social mate in the change in provisioning rate between first and second broods for those same 35 pairs, and 3) female and male provisioning rates for each nest in the dataset.

RESULTS

Environmental variability. – During the breeding seasons from 1995 – 2011, temperature, rainfall, and caterpillar biomass varied dramatically and these environmental variables differed across the elevation gradient. Temperature decreased from low to high elevations, as expected, and increased over the breeding season at each elevation (Table 1.1, Fig. 1.1a). The seasonal trends in temperature across elevations and survey periods differed by year (Table 1.1). Rainfall, in contrast, did not differ significantly among elevations (Table 1.1, Fig. 1.1a), although seasonal patterns fluctuated among years (Table 1.1). Caterpillar biomass generally increased from low to high elevations and increased over the breeding season at each elevation, with proportionally larger increases at the high elevation site (Table 1.1, Fig. 1.1b).

Path model results. – We found that the association between weather and parental provisioning was mediated by food availability (supporting *Hypothesis 1B,* but not *Hypothesis 1A*). Temperature and rainfall during the nestling stage were not directly correlated with provisioning rate as hypothesized (*Hypothesis 1A*; Appendix 1.1 Table S1, Fig. 1.2). We did find indirect associations between temperature and provisioning rate mediated by caterpillar biomass, but not adult body condition (*Hypothesis 1B*; Fig. 1.2). Both females and males delivered prey less frequently when caterpillar biomass was greater, which was associated with warmer periods (Fig. 1.2).

The hypothesized effects of weather on parental provisioning influenced offspring condition, but only via male provisioning rates (*Hypothesis 2*; Fig. 1.2). Specifically, higher male provisioning rates were associated with heavier broods (Fig. 1.2), but higher female provisioning was not significantly associated with above-average offspring condition (Path model: $Z = 0.7$, P

= 0.47, Appendix 1.1 Table S1). Lower brood mass was associated with periods of higher rainfall during the nestling stage and warmer conditions during the incubation stage (Fig. 1.2). We found no association between temperature during the incubation stage and offspring condition, after controlling for hatch date in a post-hoc analysis (Linear model: $F_{2,336} = 7.1$, $P < 0.001$; β [date] = -0.01 ± 0.003 g/ day, $t_{336} = -3.3$, $P < 0.001$; β [temperature (incubation)] = 0.0002 ± 0.01 g/ ºC, $t_{336} = 0.01$, $P = 0.99$).

Contrary to our prediction, female and male responses to environmental variability were similar (no support for *Hypothesis 3*). Female body condition was more negatively correlated with temperature than was male body condition, but both males and females had lower provisioning rates when caterpillar biomass was relatively high (*Hypothesis 3*; Table 1.2, Fig. 1.2). Female body condition during the nestling stage was lower when temperatures were warmer during the incubation stage (Fig. 1.2), even after controlling for the significant effect of capture date (Linear model: $F_{2,75} = 14.1$, $P < 0.0001$; β [date] = -0.02 ± 0.01, $t_{75} = -3.0$, $P < 0.01$; β [temperature (incubation)] = -0.06 ± 0.02, $t_{75} = -2.6$, $P = 0.01$). Rainfall and caterpillar biomass were not significantly associated with adult body condition (Appendix 1.1 Table S1, Fig. S1). Path models also indicated that environmental factors explained a greater proportion of the variance in parental provisioning by males than by females, but this difference was minor (Fig. 1.2).

Sex differences in parental provisioning. – As hypothesized, female parental investment in each brood was greater than male parental investment, which was more variable between first and second broods (*Hypothesis 4*; Fig. 1.3). Neither female nor male provisioning rates differed significantly among elevations (Kruskal-Wallis test; female: $\chi^2_2 = 0.03$, $P = 0.99$; male: $\chi^2_2 = 5.1$, $P = 0.08$; Table 1.3). Thus, we pooled provisioning data across elevations to examine sex differences in provisioning rates between first and second broods. Females delivered prey to nestlings more frequently than did their male mate for both first broods (mean difference = 0.5 ± 0.1 visits nestling^{-1}hr^{-1}, Wilcoxon signed rank test: $S_{1,322}$ = 6853.0, $P < 0.0001$; Fig. 1.3) and second broods (mean difference = 1.1 ± 0.2 visits nestling^{-1}hr^{-1}, Wilcoxon signed rank test: $S_{1,64}$ = 626.0, $P < 0.0001$; Fig. 1.3). Female provisioning rate did not differ significantly between first and second broods (Wilcoxon signed rank test: $S_{1,34}$ = -56.5, $P = 0.36$). In contrast, male provisioning rate was significantly higher for first broods than for second broods (Wilcoxon signed rank test: $S_{1,34}$ = -219.5, $P < 0.0001$). The change in provisioning rate between first and second broods was significantly greater for males than for females (male $\Delta = 0.9 \pm 0.2$ visits nestling^{-1}hr^{-1}, female $\Delta = 0.2 \pm 0.3$ visits nestling^{-1}hr^{-1}, mean difference = 0.6 ± 0.3 visits nestling^{-1}hr^{-1}, Wilcoxon signed rank test: $S_{1,34}$ = 121.0, $P < 0.05$).

DISCUSSION

While selection should favor parental investment strategies that maximize fitness returns for the amount of effort expended (Magrath and Komdeur 2003), environmental factors are theorized to affect male and female parental effort differently (Olson et al. 2008, Harrison et al. 2009, Westneat et al. 2011), leading to sexual conflict and subsequent reductions in offspring quality (Trivers 1972, 1974). For example, the value of male parental care to the survival of offspring may be higher when adverse environmental conditions affects a female's ability to compensate for reduced male care (Dunn and Robertson 1992). However, males may gain more from investing in other components of reproductive effort when environmental conditions are unfavorable (Johnsen and Lifjeld 2003, Bouwman and Komdeur 2006). Our results suggest that weather did not directly affect sex differences in parental investment, despite seasonal declines in female body condition as temperatures increased. Male and female parental investment was greatest under low resource conditions (*i.e.,* food availability), which indicates facultative adjustments in parental behavior to local resource conditions and reduced sexual conflict. However, female parental investment in each brood was greater and less variable than male parental investment, especially late in the breeding season. The flexibility in male parental investment we documented was consistent with a meta-analysis study of 193 bird species (Olson et al. 2008), which showed that adjustments in total care occurred largely due to shifts in male parental effort, because female investment was near maximum levels. In summary, food availability was an important environmental constraint favoring greater parental effort, which in turn influenced offspring condition. Below we discuss possible explanations for these findings and their ecological implications.

Food availability mediated the association between temperature and parental provisioning

Males and females occupying territories with greater food resources made fewer provisioning trips to their nests than adults on food-poor territories. Male Black-throated Blue Warblers typically deliver larger food loads than females, despite males having lower provisioning rates (Stodola et al. 2010). Greater participation in feeding by males could therefore reduce foraging pressure on females (Lee et al. 2011). Females occupying food-rich territories might also deliver larger food loads than do females on food-poor territories, which would result in fewer trips to the nest by females. The disparity between provisioning rate and food load size might explain why parental provisioning declined with increasing caterpillar biomass. In addition, male provisioning on food-rich territories may be less essential to fledge young (*e.g.,* Hoi-Leitner et al. 1999), thus enabling males more time to invest in competing breeding activities, such as extra-pair mating (Low et al. 2011). Males that occupy higher quality territories might need to allocate more time and energy to territorial defense and, as a result, reduce parental provisioning (Wingfield et al. 1990). Future studies that investigate male time budgets would help to determine how food availability influences the allocation of time to fitness-related behaviors. In particular, studying time budgets during experimental food manipulations would help to disentangle the effects of male quality from territory quality on parental investment.

The low amount of variation in parental provisioning explained by weather, caterpillar biomass, and adult body condition implies that other factors influence provisioning rates. For example, Sillett et al. (2004) investigated the effects of conspecific density on parental provisioning in *S. caerulescens* and found that females on reduced-density territories tended to provision their nestlings at a higher rate than control females in years of low food availability. Parents also reduce provisioning rates when faced with predation risk to themselves and their offspring (Ghalambor and Martin 2001, Peluc et al. 2008). Additionally, paternity may have contributed to the variation observed in male provisioning rates if males adjust their parental investment to their level of paternity, which has been documented in this species by older males (Chuang-Dobbs et al. 2001). Provisioning rates also differ between older, experienced adults and yearlings because older individuals tend to occupy higher quality, food-rich sites (Holmes et al. 1996). The comprehensive examination of these factors is beyond the scope of this study, but the effects of conspecific density, predation risk, extra-pair mating opportunity, and age on parental provisioning deserve further study.

Weather-related effects on male provisioning were correlated with offspring condition

The lower rate of male provisioning on territories with greater food resources was correlated with lighter nestlings, yet we detected no similar statistically significant relationship between lower female provisioning rate on food-rich territories and offspring condition. These results suggest that males and females use different decision rules for parental investment when the fitness benefits of parental care differ between the sexes (reviewed in Clutton-Brock 1991). Males may reduce their rate of provisioning in response to their level of paternity within a brood based on reliable cues of paternity or the opportunity for extra-pair mating at the expense of the welfare of the offspring (e.g., Hoi-Leitner et al. 1999). Females may instead reduce their provisioning rate only when they can compensate by delivering larger food loads or when the energetic requirements of young are low (*e.g.,* warmer, food-abundant conditions) and the costs to offspring are minimal (e.g., Dawson and Bortolotti 2002).

We also found direct associations between weather and offspring condition. Heavy rainfall can increase the thermoregulatory demand of adults and nestlings (Weathers et al. 2003, Johnson et al. 2007). We found that nestling mass was lower when rainfall was higher during the nestling stage. Adult foraging may have been constrained because adults did not provision their broods enough to offset increased metabolic needs of nestlings. Our results suggest that parents may have experienced a trade-off between foraging for themselves and their offspring, which might have resulted in smaller food loads and contributed to lower mean offspring condition under wetter conditions. To investigate this potential trade-off would require examining variability in food loads in addition to provisioning rates under variable environmental conditions while monitoring male and female time budgets.

Finally, studies of birds in seasonal environments have shown that breeding attempts late in the season (re-nesting or second brood attempts) often produce offspring of lower reproductive value (Perrins 1970, Verhulst and Tinbergen 1991, Drent 2006). We found that nestling mass declined as the breeding season progressed. The seasonal decline in offspring quality have been hypothesized to be a consequence of seasonal degradation of environmental conditions that produce constraints on foraging by parents, parental investment, and offspring growth (Verhulst and Nilsson 2008). However, our data do not support this explanation because environmental conditions improved over the breeding season. Lower quality offspring could be produced later in the season by lower quality parents (*e.g.,* younger and less experienced breeders, poor body condition) or parents occupying lower quality territories that initiated breeding later (reviewed in Verhulst and Nilsson 2008). Alternatively, the survival of young fledged early in the season may depend on food reserves because temperatures are cooler. Late in the season when temperatures are warm, the energetic costs are lower lessening the dependence on stored reserves (body mass) to achieve the same level of survival.

Females and males responded similarly to environmental variability

Although we found no sex-specific parental responses to environmental variability, females consistently provisioned nestlings at a higher rate than did males despite seasonal mass loss. Females were also much less variable in their parental investment in each brood compared to males. The finding that temperature during the incubation stage was associated with female body condition during the nestling stage is likely an artifact of the strong correlation between temperature and timing of breeding, as well as female body condition and timing of breeding. Female body condition declines during the breeding cycle in a number of species, likely because they invest in reproductive behaviors, such as incubating and foraging for nestlings, at the expense of maintenance of energetic reserves (Nagy et al. 2007). Females that initiate nests earlier in the season may have been of higher quality (*i.e.* heavier) compared to later nestling females, which could partially explain the decline in female body condition with the timing of breeding (Nagy et al. 2007).

Conclusions and Implications

In summary, we found that: 1) food availability mediates the association between temperature and parental investment, 2) individual variation in male parental investment is correlated with offspring condition, 3) both sexes respond similarly to environmental variability, and 4) female parental investment in each brood is greater and less variable than male parental investment, likely accounting for the observed seasonal declines in female body condition. Our study indicates that birds adjust their reproductive decisions based on territory quality, such that birds increase their level of parental care when food resources are low, which is generally associated with cooler conditions. Thus, the association between temperature and caterpillar availability is an important environmental constraint favoring greater parental effort in Black-throated Blue Warblers, potentially at the expense of other components of reproductive effort. Studies that examine individual variation in parental effort in response to experimental manipulations of temperature (Ardia et al. 2009) and food availability, a critical factor limiting annual fecundity and adult survival (Rodenhouse and Holmes 1992, Sillett et al. 2000, Nagy and Holmes 2005), are especially timely to predict the effects of climate-related changes in habitat quality (*i.e.,* food availability) on the reproductive fitness of migratory songbirds.

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TABLE 1.1. Results from univariate GLMs used to examine how temperature, rainfall, and caterpillar biomass varied seasonally and annually at each elevation during the Black-throated Blue Warbler breeding seasons from 1995 - 2011 at the Hubbard Brook Experimental Forest, NH.

Variable	df	*F*	*P*
Temperature			
Elevation	2, 96	633.8	< **0.0001**
Year	16, 96	96.7	< **0.0001**
Survey period	3, 96	612.7	< **0.0001**
Year x survey period	48, 96	44.1	< **0.0001**
Elevation x year	32, 96	8.3	< **0.0001**
Elevation x survey period	6, 96	1.3	0.28
Rainfall			
Elevation	2, 96	0.3	0.76
Year	16, 96	383.1	< **0.0001**
Survey period	3, 96	440.5	< **0.0001**
Year x survey period	48, 96	86.4	< **0.0001**
Elevation x year	32, 96	0.8	0.78
Elevation x survey period	6, 96	0.4	0.88
Caterpillar biomass			
Elevation	2, 96	12.6	< **0.0001**
Year	14, 96	72.5	< **0.0001**
Survey period	2, 96	21.8	< **0.0001**
Year x survey period	15, 96	8.0	< **0.0001**
Elevation x year	8, 96	16.7	< **0.0001**
Elevation x survey period	2, 96	3.2	**0.04**

Bold type highlights significant model effects ($P < 0.05$).

TABLE 1.2. Tests of equality of path coefficients from models used to assess sex differences in response to temperature, rainfall, and caterpillar biomass by *S. caerulescens* at the Hubbard Brook Experimental Forest, NH. Results from the path models are unstandardized path coefficients for all factors, significance values, and the proportion of variance explained by each factor in the model. Significant factors are in bold.

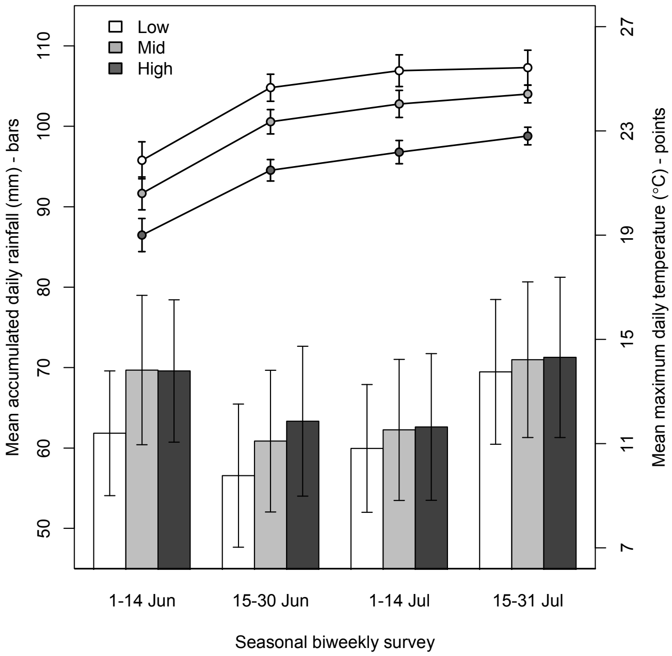
		Female path model				Male path model				Tests of equality		
Response variable	Explanatory variable	β ± SE	*Z*	*P*	R^2	β ± SE	*Z*	*P*	R^2	*df*	*t*	*P*
Adult body condition	Temperature (incubation)	-0.09 ± 0.02	-4.7	< **0.001**	0.23	0.02 ± 0.01	1.4	0.15	0.03	125	3.9	< **0.0001**
	Caterpillar biomass	-0.03 ± 0.04	-0.7	0.49		0.01 ± 0.03	0.3	0.78		125	0.7	0.51
Provisioning rate	Temperature (nestling)	-0.02 ± 0.03	-0.8	0.46	0.02	-0.03 ± 0.02	-1.4	0.16	0.04	123	-0.4	0.66
	Rainfall (nestling)	-0.003 ± 0.003	-1.1	0.26		0.002 ± 0.002	0.7	0.50		123	1.3	0.21
	Caterpillar biomass	-0.13 ± 0.05	-2.7	< **0.01**		-0.11 ± 0.04	-2.8	< **0.01**		123	-0.1	0.90
	Adult body condition	0.02 ± 0.20	0.1	0.91		-0.19 ± 0.29	-0.7	0.51		123	-0.6	0.57

TABLE 1.3. Parental provisioning rate[a] by female and male *S. caerulescens* on day 7 of the nestling stage and offspring condition[b] on day 6 of the nestling stage from first and second broods across elevations at the Hubbard Brook Experimental Forest, NH, 1995-2011. Values are given as means ± SE and number of nests provided in parentheses.

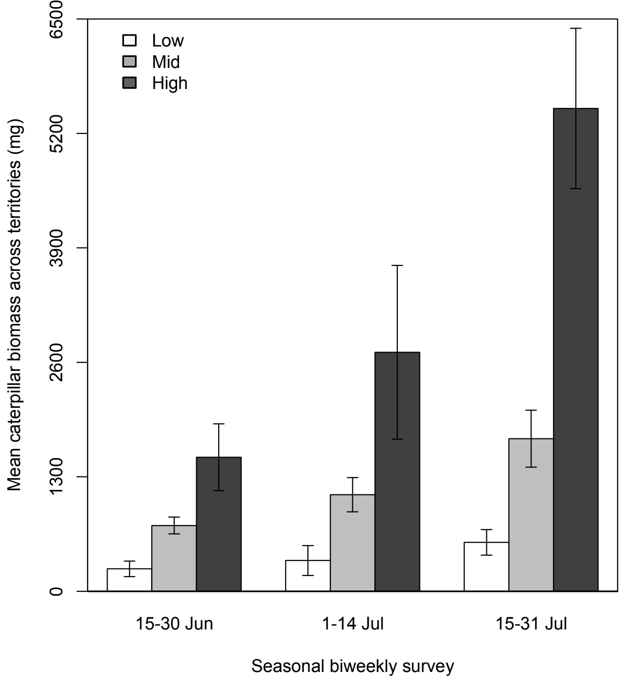
	Low elevation		Mid elevation		High elevation	
	First	Second	First	Second	First	Second
Female provisioning rate	2.3 ± 0.3 (40)	1.5 ± 0.5 (2)	2.1 ± 0.1 (211)	2.0 ± 0.2 (44)	2.0 ± 0.2 (30)	2.4 ± 0.3 (19)
Male provisioning rate	1.5 ± 0.2 (40)	0.6 ± 0.6 (2)	1.7 ± 0.1 (211)	1.1 ± 0.2 (44)	1.5 ± 0.1 (75)	0.9 ± 0.2 (19)
Offspring condition	7.9 ± 0.1 (40)	7.5 ± 0.3 (2)	7.9 ± 0.1 (211)	7.8 ± 0.1 (44)	8.0 ± 0.1 (75)	7.5 ± 0.2 (19)

[a] Provisioning rate is the number of provisioning visits to the nest per nestling per hour

[b] Mean mass of broods (g)



(a) Rainfall and temperature patterns at 3 elevations



(b) Index of caterpillar availability at 3 elevations

Figure 1.1. Patterns of rainfall and temperature (a) and caterpillar biomass (b) at low, mid, and high elevation study plots at the HBEF. Mean (± SE) accumulated daily rainfall (mm) on the left axis (bars) and maximum daily temperature (°C) on the right axis (points) was measured at weather stations, 1995–2011. Caterpillar biomass (mg) was estimated within *S. caerulescens* territories, 1997–2011. All data were averaged by biweekly surveys during the breeding season (June-July) across years.

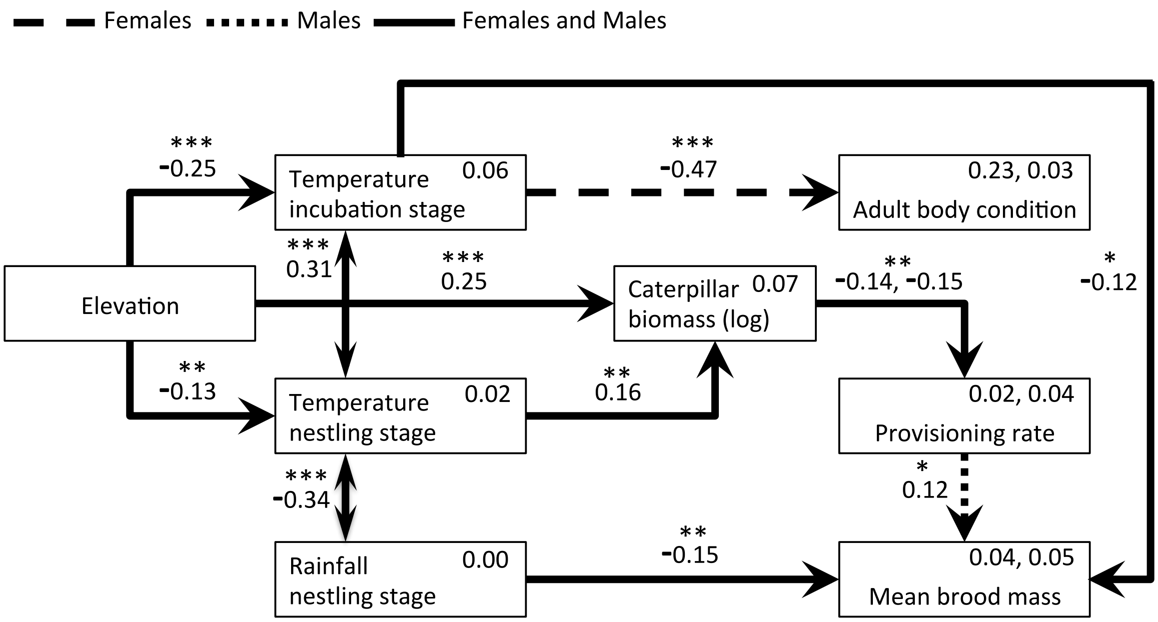


Figure 1.2. Path model testing hypothesized direct and indirect effects of environmental conditions on adult body condition and nestling provisioning rates by female and male *S. caerulescens* and resulting effects on offspring condition at the Hubbard Brook Experimental Forest, NH. Environmental conditions include mean maximum daily temperature during the incubation and nestling stage, accumulated daily rainfall during the nestling stage, and caterpillar biomass sampled near the time of parental provisioning. Significant ($P < 0.05$) paths are delineated with arrows; solid arrows indicate paths significant both for females and males, dashed arrows for females only, and dotted arrows for males only. Values next to arrows are standardized path coefficients. The proportion of variance explained by each factor in the path model appears above the name of that factor in the box, with left values for females and right values for males. Only significant paths are shown; the full model is provided in Appendix 1.1, Figure S1. Path model support: female $\chi^2_{11} = 8.4$, $P = 0.68$, RMSE < 0.0001, CFI = 1.00; male: $\chi^2_{11} = 11.0$, $P = 0.45$, RMSE < 0.0001, CFI = 1.00.

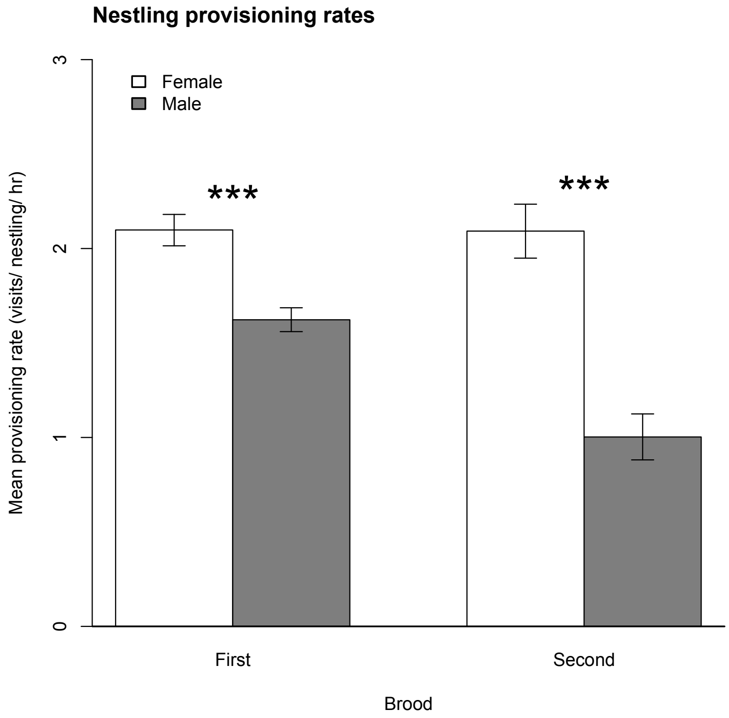
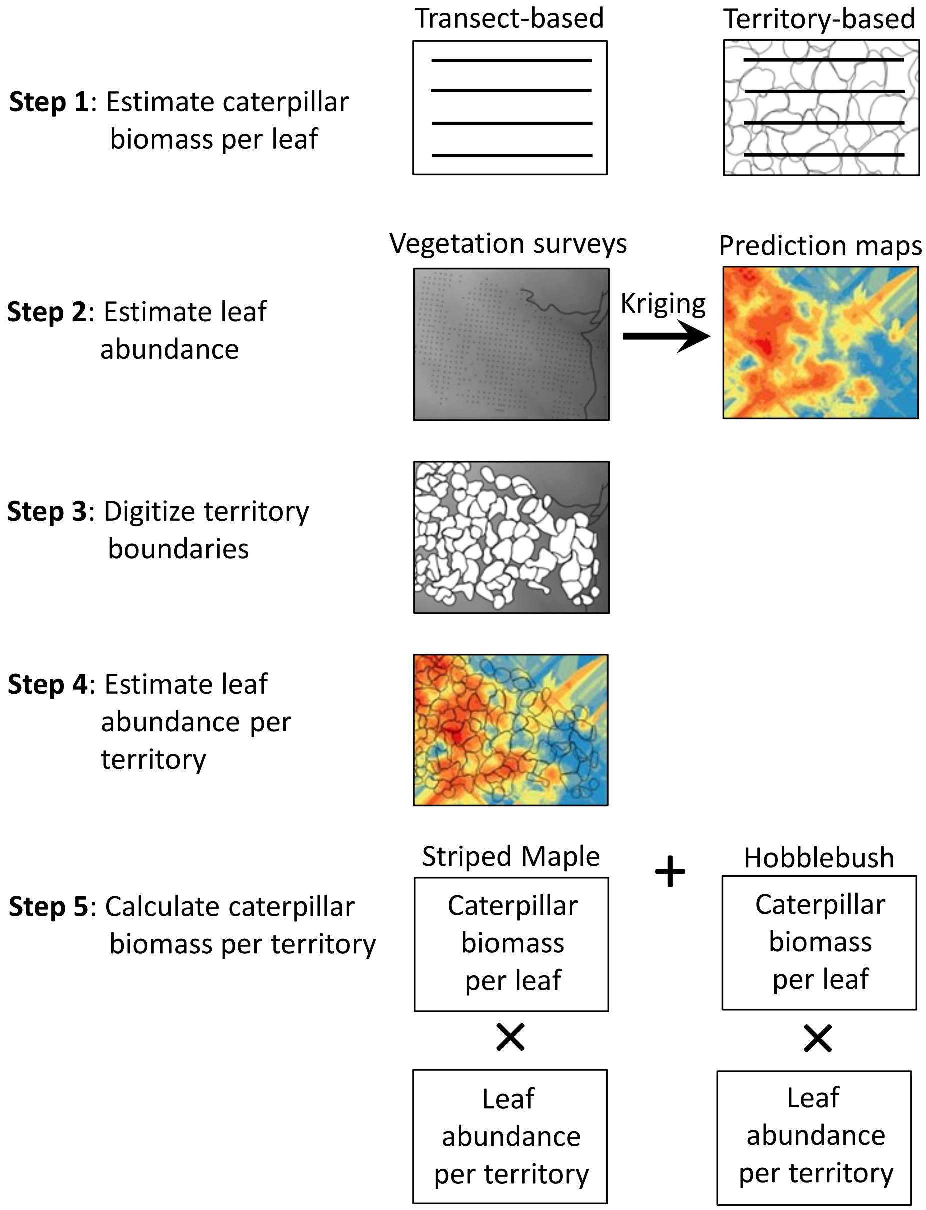


Figure 1.3. Nestling provisioning rates of female and male *S. caerulescens* to first and second broods (mean ± SE for the three elevations combined) at the Hubbard Brook Experimental Forest, NH. Paired-sample mean comparisons (Wilcoxon signed rank test) were between females and males for first and second broods (****P* < 0.001).

APPENDIX 1.1. Methods used to generate the index of caterpillar biomass per territory.



Step 1. Estimate caterpillar biomass per leaf

We estimated caterpillar biomass per leaf along plot-wide transects (2000-2002, 2007-2011) or within individual territories (1997-1999, 2003-2006). In each year, we conducted four biweekly surveys within the breeding season (1 June – 31 July), beginning after leaves in the understory had fully expanded.

For plot-wide surveys, we sampled 10 points separated by 50 m along 4 transects spanning the central area of each study plot. Surveys were centered at each sampling point, and we visually searched for caterpillars on 100 striped maple leaves and 100 hobblebush leaves located within 25 m of the point. For this study, only caterpillar surveys on striped maple and hobblebush foliage are presented, because 1) striped maple and hobblebush were censused in each year, 2) patterns of estimated caterpillar biomass across the four biweekly surveys were similar between these two species, and; 3) estimated caterpillar biomass during each biweekly survey contained non-zero data. We measured the body lengths of caterpillars to the nearest millimeter and converted the number and length (l) of the caterpillars into estimated wet biomass (b) in units of mg caterpillar biomass using the formula: $b = 0.004 \text{ x } l^{2.64}$ (Rogers et al. 1977). To obtain unique estimates of caterpillar biomass per leaf for each plant species in each survey of each year, we averaged per-transect estimates of caterpillar biomass across the four transects and divided by 1000 leaves. We did not conduct surveys in 1995, 1996, or in 2002 on the low elevation study plot.

The territory-based surveys of caterpillars were centered at a nest site or a random point within individual territories when a nest was not available. We visually searched for caterpillars on 50 striped maple leaves and 50 hobblebush leaves located 10 m and 15 m from the point in each of the four cardinal directions. To obtain unique estimates of caterpillar biomass per leaf for each plant species in each survey of each year, we averaged estimates of caterpillar biomass per territory across the subset of territories located within the area surveyed by plot-wide transects and divided by 400 leaves. Restricting the estimation of caterpillar biomass per leaf to these territories overlapping established transects minimized potential differences between territory and transect-based estimates of caterpillar biomass. Our survey methods did not account for variation in caterpillar detectability and, thus, should be considered an *index* of caterpillar biomass per leaf (*i.e.,* food available to birds).

Step 2. Estimate leaf abundance

Leaf abundance was sampled from 1997-2001 and from 2007-2009 in late July after leaves had fully expanded. Sampling points were located across each study plot (low = 123, mid = 668, high = 143) and each separated by ≥50 m. Centered at each sampling point, a vertical 9-m^2 plane was created using two poles 3 m in height and connected by a 3 m chain located 11.2 m from the point in each cardinal direction (Sillett et al. 2004). To obtain an index of leaf abundance between 0-3 m in the understory per point, we counted the number of leaf intersections within each of the four 9-m^2 planes for striped maple and hobblebush, and summed them.

To estimate leaf abundance, we used ordinary kriging to interpolate leaf counts of striped maple and hobblebush for each study plot and produced prediction maps of leaf abundance for each plant species by plot using the Geostatistical Analyst extension of ArcGIS 10 (ESRI 2011). We used the mean of the samples for coincidental points sampled in more than one year. We used the Matérn semivariogram model to estimate the covariance structure and we specified that the leaf abundance data were measured with uncertainty. To estimate local means, we used a search neighborhood of the nearest 12 sampling points encompassed within a 100 m radius. Output cell sizes differed among study plots due to differences in the initial spatial coverage of the sampling points (low = 3.3-m^2, mid = 5.7-m^2, high = 2.1-m^2). For each plot, output cell sizes fit within the territory polygons, improving the accuracy of estimations of leaf abundance per territory.

Step 3. Digitize territory boundaries

To digitize territory boundaries, we collected GPS coordinates of plot grid points. We georeferenced images of territory maps to these plot grids using a minimum of 10 control points and digitized territory polygons for each year.

Step 4. Estimate leaf abundance per territory

Last, we obtained the leaf abundance estimates of each plant species for each territory using the isectpolyrst tool in the Geospatial Modelling Environment (Beyer 2012), that generated sum of leaves for all raster cells contained within each territory polygon based on the values in the kriged raster layers.

Step 5. Calculate caterpillar biomass per territory

To estimate caterpillar biomass within individual territories in each survey period, we multiplied estimates of caterpillar biomass per striped maple and hobblebush leaf by the abundance of leaves within territories and summed each value. For the index of caterpillars available to provisioning adults for each nest, we used the estimate from the survey period coinciding with the nestling stage of that nest.

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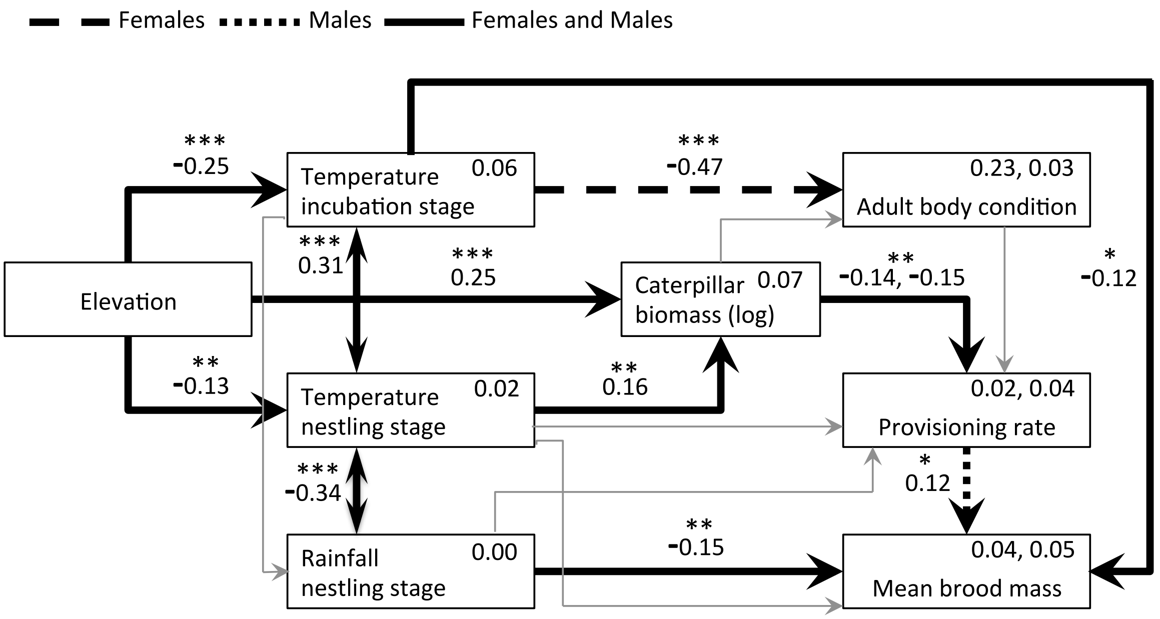

Figure S1. Best-supported full path model testing hypothesized direct and indirect effects of environmental conditions on adult body condition and nestling provisioning rates by female and male *S. caerulescens* and resulting effects on offspring condition at the HBEF. Environmental conditions include mean maximum daily temperature (incubation and nestling stage), accumulated daily rainfall (nestling stage), and caterpillar biomass. Significant ($P < 0.05$) paths are delineated with arrows; solid arrows indicate paths significant both for females and males, dashed arrows for females only, dotted arrows for males only, and gray arrows for insignificant paths. Asterisks indicate significance levels (***$P < 0.001$, **$P < 0.01$, * $P < 0.05$). Values next to arrows are standardized path coefficients and the proportion of variance explained by each part of the path model appear above the name of each response variable in the box with left values for females and right values for males.

TABLE S1. Path analysis examining effects of temperature, rainfall, and caterpillar biomass on adult body condition and nestling provisioning rates by female and male *S. caerulescens* and resulting effects on offspring condition at the HBEF. Results include total effects (standardized coefficients) for all factors (see Fig. 3 for direct and indirect effects). Significant factors are in bold (***$P <$ 0.001, **$P < 0.01$, * $P < 0.05$). Model fit: $\chi^2_{11} =$ 8.4, $P = 0.68$, RMSE < 0.0001, CFI = 1.00 (female); $\chi^2_{11} =$ 11.0, $P = 0.45$, RMSE < 0.0001, CFI = 1.00 (male). Full model provided in Appendix 1.1, Figure S1.

Model	Elevation	Mean maximum temperature (incubation)	Mean maximum temperature (nestling)	Rainfall sum (nestling)	Caterpillar biomass	Adult body condition	Provisioning rate
Female							
Temperature (incubation)	**-0.253** ***						
Temperature (nestling)	**-0.130** **						
Caterpillar biomass	**0.227** ***		**0.153** **				
Adult body condition	0.103	**-0.473** ***	-0.012		-0.076		
Provisioning rate	-0.025	-0.004	-0.063	-0.060	**-0.141** **	0.009	
Mean brood mass	0.019	**-0.121** *	0.078	**-0.145** **	-0.006	0.000	0.039
Male							
Temperature (incubation)	**-0.253** ***						
Temperature (nestling)	**-0.130** **						
Caterpillar biomass	**0.222** ***		**0.159** **				
Adult body condition	-0.033	0.157	0.005		0.032		
Provisioning rate	-0.021	-0.011	-0.099	0.036	**-0.152** **	-0.069	
Mean brood mass	0.016	**-0.118** *	0.072	**-0.148** **	-0.017	-0.008	0.115 *

CHAPTER 2

REPRODUCTIVE BEHAVIOURAL AND HORMONAL PLASTICITY IN RESPONSE TO EXPERIMENTAL FOOD SUPPLEMENTATION AND HABITAT QUALITY IN A MIGRATORY SONGBIRD*

*** S. A. Kaiser, T. S. Sillett, and M. S. Webster**

Summary

1. Behavioural plasticity can allow organisms to adjust to changing environmental cues, such as food availability, that regulate reproduction. However, adaptive behavioural plasticity in response to changing resource conditions will depend how food affects the underlying endocrine mechanisms generating behavioural variation.
2. We examined how food availability and endocrine physiology interact to regulate reproductive effort of black-throated blue warblers (*Setophaga caerulescens*) at the Hubbard Brook Experimental Forest, New Hampshire, USA, 2009 – 2012. We provided supplemental food to free-living adults and hypothesized that reduced energetic constraints due to increased food would affect 1) the relative allocation of male reproductive effort to mating and parental behaviours, 2) the hormones regulating male reproductive behaviours, and 3) female parental effort.
3. The effects of supplemental feeding were more pronounced in low quality habitat. Food supplementation influenced male song rate but not parental behaviour. Fed males sang at lower rates near the nest site during their social mate's fertile stage, suggesting increased mate guarding effort relative to control males. Fed males in low quality habitat had lower plasma androgens during the fertile stage than control males. Males with high plasma androgens sang at high rates, were in poor body condition, and had high baseline corticosterone (CORT). Fed males also appeared to benefit indirectly from access to supplemental food because fed females provided more parental effort than did control females. During their social mate's fertile stage, fed males in both low and high quality habitat were more able to meet their energetic needs relative to control males as indicated by their better body condition and lower baseline CORT. Fed males, however, had higher

baseline CORT during the parental stage than control males, possibly because fed males invested greater effort in extra-pair mate attraction at that time. Parental effort was not associated with androgen or baseline CORT concentrations.

4. These adjustments in mating and parental effort allow birds to respond to changes in resource conditions that constrain reproduction, and they suggest a role for food in mediating hormone-regulated reproductive effort.

Introduction

Behavioural plasticity can buffer populations against environmental change and facilitate adaptive evolution (Ghalambor et al. 2007, Duckworth 2009). Such plasticity depends on both the environmental cues and the physiological mechanisms that animals use to regulate reproduction (Dawson 2008, Lessells 2008). Seasonal changes in plasma hormone levels can modulate reproductive behaviours that influence fitness (Adkins-Regan 2005), but the evolutionary constraints on these endocrine mechanisms is poorly understood. Specifically, how environmental cues, such as food, affect endocrine systems. A mechanistic understanding of reproductive behavioural plasticity in response to food limitation is needed to determine whether adaptive behaviour is sufficient to compensate for environmental change (Adkins-Regan 2008, Wingfield 2008). Studies of birds have been especially valuable in understanding how the environment influences relationships among hormones and behaviour (Adkins-Regan 2005).

Birds use hormonally-mediated breeding strategies to adjust reproductive effort to resource conditions (Hau 2007). Food availability can influence the allocation of effort to mating (Hoi-Leitner et al. 1999, Forstmeier 2003, Václav et al. 2003), parental care, and self-maintenance behaviours (e.g., Low et al. 2006). Testosterone (T), which influences these competing reproductive behaviours, has a central role in regulating trade-offs between mating and parental effort in male birds (Wingfield et al. 1990, Ketterson et al. 1996, Adkins-Regan 2005). Hormone manipulations have demonstrated a causal link between T and sexual behaviours (reviewed in Fusani 2008). The suppression of paternal behaviour by T, however, has received mixed support (e.g., Lynn et al. 2002). Recent correlative evidence also demonstrates a potential role for glucocorticoids in regulating parental investment (Jenni-Eiermann et al. 2008). Elevated corticosterone (CORT) affects behaviours that promote adult survival (reviewed in Wingfield and Ramenofsky 1999), at a cost to parental care of nestlings (Silverin 1998, Almasi et al. 2008). Baseline CORT concentrations could be direct indicators of energetic stress (*i.e.,* body condition) and thus predict the likelihood of survival and reproductive success relative to local resource conditions (Schoech et al. 2007, Herring et al. 2011). Testosterone and CORT interact to regulate reproductive trade-offs between mating and parental effort (Hau 2007, Hau et al. 2010), which indicates the importance of measuring both hormones in studies of reproductive behaviour. Although these hormones are likely involved in the behavioural mechanism regulating plasticity in reproductive trade-offs, such plasticity will depend on the sensitivity of these mechanisms to environmental cues (Dawson 2008).

The optimal expression of reproductive behaviours in response to changes in environmental conditions depends on the translation of environmental stimuli into hormone secretions (Wingfield 2008). Most studies have focused on the effects of social stimuli on hormonal responses, but less empirical work has investigated the importance of non-social environmental cues, such as food availability, to inform hypotheses about the sensitivity of endocrine mechanisms to these cues (Goymann et al. 2007, Kempenaers et al. 2008). However, a few studies have demonstrated the effects of supplementary environmental cues, such as food, on the hormones regulating the onset of reproduction in seasonally breeding birds at temperate latitudes (e.g., Schoech 2009). We are aware of no study, however, that has examined the plasticity in reproductive effort due to the effects of food on the hormones that regulate mating and parental behaviours once breeding is under way. Yet, this supplementary environmental cues can depress the secretion of circulating T and CORT, that regulate the expression of reproductive behaviours (Hahn 1995, Jenni-Eiermann et al. 2008). Determining whether environmental cues mediate hormone-regulated reproductive effort under natural conditions will aid in our understanding the capacity of birds to respond adaptively to predicted changes in food availability (Goymann et al. 2007, Wingfield et al. 2008).

Here, we investigate the behavioural and hormonal plasticity of a migratory songbird, the black-throated blue warbler (*Setophaga caerulescens,* Fig. 2.1) in response to food availability. Our research focuses on the role of T and CORT in regulating male reproductive trade-offs on food-supplemented territories versus control territories in habitats with low and high food abundance at the Hubbard Brook Experimental Forest (HBEF), New Hampshire USA. The abundance of *Lepidoptera* larvae, a major food source of black-throated blue warblers (Holmes et al. 2005), and hence territory quality, is positively correlated with elevation at the HBEF (Chapter 1). We hypothesized that reduced energetic constraints due to increased food would affect: 1) the relative allocation of male reproductive effort to mating and parental behaviours, 2) the hormones regulating male reproductive behaviours, and 3) female parental effort. We predicted that, relative to controls, fed males would invest more into mating effort and less into parental effort, be in better body condition, and maintain higher plasma T and lower baseline CORT during both their social mate's fertile stage (egg-laying) and periods of bi-parental care. We predicted that fed females would invest more in parental effort relative to control females, which in turn would reduce the amount of parental effort required from their respective social mates. We then examine if habitat quality mediated the effects of food supplementation on behavioural and hormonal responses. We predicted that supplemental feeding would significantly affect these responses in low quality habitat, and that supplemental feeding would have a reduced or negligible effect in high quality habitat.

Selection on adaptive hormone-mediated reproductive trade-offs between mating and parental effort in response to environmental variation should occur at the level of the individual (Eikenaar *et al.* 2011). We therefore examine individual variation in the relationships between hormones and male reproductive behaviours. First, we tested the hypothesis that T mediates male reproductive trade-offs between mating and parental effort. We predicted that: 1) T concentrations would be higher in males during their social mate's fertile stage than during the parental stage, 2) higher T concentrations would be positively associated with mating effort, and 3) higher T concentrations would be negatively associated with parental effort. Second, we tested the hypothesis that CORT indicates energetic stress, measured by body condition, and is predictive of parental effort. We predicted that elevated baseline CORT concentrations in males would be associated with both poor body condition and reduced parental effort.

Materials and Methods

Study population

We studied a marked population of black-throated blue warblers breeding in the 3160-ha HBEF, Woodstock, New Hampshire, USA (43°56'N, 71°45'W), an intact northern hardwood forest. We collected data over a 4-yr period (May-August, 2009-2012) on three, gridded study plots previously established at low (250-350 m; 85 ha), mid (450-600 m; 65 ha), and high (750-850 m;

35 ha) elevations. We grouped data by habitat quality (low quality = low elevation plot, high quality = mid and high elevation plots) because natural variation in food availability, as well as, reproductive output at the mid elevation plot were, on average, similar to that on the high elevation plot, but differed substantially from the low elevation plot (N.L. Rodenhouse, unpublished data). All black-throated blue warblers were identifiable with a unique combination of three coloured leg bands and an aluminum U.S. Geological Survey leg band. The black-throated blue warbler is a socially monogamous, Nearctic-Neotropical migratory songbird. The HBEF population has relatively high rates of extra-pair paternity (34%, 1995-1998) (Webster et al. 2001). Males guard fertile females during the nest building and egg-laying stages, following females closely while singing (Holmes et al. 2005). Females incubate (mean clutch size = 3.6, range = 2–5 eggs) without assistance from males for *ca.* 12 days, but both sexes feed nestlings for *ca.* 9 days until fledging (see Holmes et al. 2005 for more information on their breeding biology). Adults are insectivorous and deliver mostly Lepidoptera larvae (*ca.* 90% of nestling diet) to nestlings (Rodenhouse and Holmes 1992, Goodbred and Holmes 1996). Pairs will sometimes attempt second broods after fledging first broods, with the probability of double-brooding increasing with food availability (Nagy and Holmes 2005a). During each breeding season, we mapped male territories and monitored all nest attempts.

Field methods

Following territory establishment, we arbitrarily assigned 6 - 8 territories per study plot to the food-supplemented (hereafter, ‘fed’) treatment and designated 15 – 20 territories per study plot as controls. Fed and control territories were separated by at least one control territory to reduce the probability of control males finding and using feeding trays. We typically found nests during building and began supplemental feeding of first nest attempts 2 – 3 days after the onset of incubation to avoid affecting lay dates or causing nest abandonment.

We initially placed feeding trays 1 m from nests at nest height and then moved them further from the nest (~5 m) once we visually confirmed that the female and/or male was regularly feeding from the tray. Details on the design of the feeding tray are described in Nagy and Holmes (2005). We conducted daily observations at the feeding trays to determine whether other species were taking food from the trays (*e.g.,* small mammals and other songbirds foraging in the understory). If other species were detected feeding from the tray, we moved the tray to a new location near the nest. We reassigned fed treatments after four days had passed without a female taking food. When a nest failed, we paused food delivery until the new nest was found and began feeding at the new nest once the females began incubating their new clutch. When a nest fledged, we continued to provide food at the tray until food was no longer being taken (*i.e.,* fledglings dispersed) or moved the tray to the new nest if a second brood was initiated.

We adjusted the amount of food provided based on the nest stage. We initially provided 5 g of waxmoth larvae (Lepidoptera: *Galleria mellonella*). Once females were documented feeding, we increased the amount of food delivered to 7 g (52 kJ), which included 2 g of waxmoth larvae and 5 g of mealworms (Coleoptera: *Tenebrio monitor*) that we had gut-loaded with cricket meal to increase protein and calcium content. After nestlings hatched, we increased the amount of food delivered to 14 g (104 kJ), 10 g of mealworms and 4 g of waxmoth larvae, because adults regularly provided larvae to their young. We delivered new food daily and removed uneaten larvae from trays. We varied the time of day that food was delivered so that birds did not learn to anticipate food, which could affect baseline hormone levels (Reneerkens et al. 2002).

In analyses examining the effects of the feeding treatment, we only included fed nests where we had confirmed that adults were feeding from the tray for one complete nest stage (*e.g.,* fed during incubation stage or nestling stage) prior to blood or behavioural data collection. The sample sizes for nests provided supplemental food differed between the fertile (egg-laying) and parental stages (nestling days 3-7) because: 1) supplemental feeding of first nests was initiated a few days after the onset of incubation, limiting sample sizes during the fertile stage (*i.e.,* re-nesting attempts, second broods); 2) adults differed in when they began feeding from the trays following the establishment of feeding trays; 3) nests were found at different nest stages; and 4) nests failed at different nest stages due to abandonment, weather, and predation. We observed males and females regularly using feeding trays at 14 nests in 2009 (low quality habitat = 7, high quality habitat = 7), 35 nests in 2010 (low = 6, high = 29), 32 nests in 2011 (low = 11, high = 21) and 27 nests in 2012 (low = 5, high = 22). In total, we successfully fed 11 pairs in 2009, 21 pairs in 2010, 18 pairs in 2011, and 18 pairs in 2012, for which we collected data from multiple nests on individual territories.

We captured males during the egg-laying stage when their social mates were fertile (‘fertile stage’) and attempted to recapture them during the nestling stage (‘parental stage’). Males were lured into mist nets with song playback and a male decoy (‘simulated territorial intrusion’, STI) between 0600 and 1400. We recorded the time elapsed between the initiation of playback and capture (‘response latency’) and the time elapsed between capture and blood sampling (‘bleed time’). Within 5 min of capture (mean = 3.0 ± 0.2 min), we obtained blood samples by puncturing the brachial vein with a small (26 G) needle and collected approximately 80 µl of whole blood from the *ca.* 10 g males into heparinized microcapillary tubes. Blood samples were stored on ice in the field and centrifuged within 6 hours of collection. We transferred the plasma into microcentrifuge tubes and stored the samples at -20°C until we performed hormone assays.

At capture, we took morphometric data to age warblers and assessed male body condition. We aged individuals as second-year (SY) or after second-year (ASY) using plumage characteristics (Pyle *et al.* 1997) and measured the length of the right tarsus to the nearest 0.01 mm and mass to the nearest 0.1 g. We indexed male body condition using the size-corrected body mass from the residuals of a linear regression of mass on tarsus length (Linear regression: $F_{1,433} = 13.8$, $P < 0.001$, $R^2 = 0.03$).

Male birds spend considerable time and energy singing to attract and guard mates, and to defend their territories (Kroodsma and Byers 1991, Nowicki and Searcy 2005). We used song rate, which is correlated with body condition and can reflect resource limitation (Grava et al. 2009), as our index of male mating effort. Higher song rate would indicate greater allocation to mating effort. To measure song rate, we placed digital voice recorders (Olympus DS-30) within 10 m of nests, 2010-2012. The recorders were capable of detecting songs within a radius of 60 m from the stereo microphone. They were automated to record for three 10-min periods at 0530, 0600, and 0630 on each of four consecutive mornings during a female's fertile stage (egg-laying) and parental stage (nestling days 3-7). We generated song rates from recordings using the band-limited energy detector in Raven 1.4 (BRP 2004). See Appendix 2.1 for validation details. Male song rate was calculated by averaging the number of songs detected per minute across the three morning sampling periods (30 min total) on each of the four sampling days for the fertile (3-4 egg-laying days) and parental stages (4 days, nestling days 3-7) separately.

We extracted three measures of female and male parental effort – nestling provisioning rate, the amount of prey biomass delivered, and time spent at the nest – from 2-hr video recordings of nests collected after dawn on day 7 of the nestling stage. Video cameras (*e.g.,* JVC Everio GZ-MG155U) were mounted on tripods, set at the height of the nest rim, and placed 10 m from nests. For each nest visit, we confirmed the colour band combination of the feeding parent, and recorded its arrival time, the number and type of prey items (*Lepidoptera* larvae versus adult insects) brought to the nest and their size relative to the length of the exposed bill (~7 mm), and departure time. Prey were grouped into four length classes: <7 mm, 7-14 mm, 14-21 mm, and >21 mm. We converted the number and length (l) of the prey items into estimated prey biomass (b) in units of mg prey biomass for each nest visit using the formula: $b = 0.004 \times l^{2.64}$ (Rogers et al. 1977). We standardized each hourly measure of parental behaviour to per nestling rates by dividing by brood size. Note that results did not differ when we did not standardize data for broods size (results not shown)

Hormone assays

We obtained 10 to 50 μL of plasma from males during both their social mate's fertile stage and the parental stage; these collections were separated by the incubation stage, and so were >12 days apart. We validated and used a commercially available testosterone enzyme immunoassay kit (Cat. #1-2402, Salimetrics LLC, State College, PA) to measure total androgen concentrations of plasma samples. The testosterone antibody cross-reacts 100% with testosterone, 36.4% with dihydrotestosterone, 21.0% with 19-nortestosterone, 1.9% with 11-hydroxytestosterone, 1.2% with androstenedione, and less than 1% for all other steroids (values provided by manufacturer). We followed the manufacturer's protocol except that we diluted 7 μL of plasma samples at 1:10 with steroid dilutant according to Washburn et al. (2007). We reran samples >600 pg mL^{-1} at a 1:40 dilution. The validation included parallelism and recovery of exogenous androgens on five pooled plasma samples. Each pool contained plasma from individuals expected to have high (territory establishment) and low (post-breeding) endogenous concentrations. Serial dilutions (1:10, 1:20, 1:40, 1:80) of pooled plasma samples yielded a displacement curve that was parallel in slope to the standard T curve. Mean recovery of exogenous T added to the high and low pooled plasma samples was 102.6% and 111.9%, respectively. We randomly distributed samples from different nest stages across the twelve assays. The intra-assay (n = 5) coefficient of variation (CV) was 1.44% and the inter-assay CV was 4.50% (n = 8 assays; 2009-2011 samples) and 9.56% (n = 4 assays; 2012 samples). The sensitivity of the assay was 15 pg mL^{-1} (Washburn *et al.* 2007).

We validated and used a commercially available radioimmunoassay kit (Cat. #07120102, MP Biomedicals, Orangeburg, NY) to measure baseline CORT concentrations of plasma samples. We followed the manufacturer's protocol except that we miniaturized the assay by halving the volumes of reagents and by diluting 3 μL of plasma samples at 1:50 with the assay dilutant according to Washburn *et al.* (2002). The validation included parallelism and recovery of exogenous CORT that was added to a low plasma pool. The low pool was derived from five birds that were bled within 3 min of capture. Mean recovery of exogenous CORT added to the low-pooled plasma sample was 99.5%. The high pool contained plasma from birds evaluated in a previous study that involved an injection of gonadotropin releasing hormone and then confinement in a cloth bag for 20 min. Serial dilutions (1:25, 1:50, 1:100, 1:200) of the high-pool plasma sample yielded a displacement curve that was parallel in slope to the standard CORT curve. We randomly distributed samples from different nest stages across the six assays. The intra-assay (n = 5) CV was 5.12% for the low pool and 6.80% for the high pool and the inter-assay (n = 6 assays; 2009-2012 samples) CV was 8.60%. The sensitivity of the assay was 3.1 ng mL^{-1}

Statistical analyses

We examined whether model assumptions were met prior to statistical analyses and grouped data when necessary when sample sizes were limited. We checked for normality with plots of model residuals and we tested for equality of variances with Levene's test. We transformed data to improve the normality of the model residuals as necessary, and used non-parametric tests when we could not meet the normality assumption. We pooled hormone and behavioural data from all years because of low sample sizes of fed males, but included categorical year as a fixed effect in models when appropriate. We grouped data by habitat quality (low quality = low elevation plot, high quality = mid and high elevation plots) as justified in "Study population". All analyses were performed using R version 2.15.3 (R Core Development Team 2013). We present results as untransformed means ± SE.

We examined differences in reproductive effort between food supplemented and control birds in relation to habitat quality. We examined the effect of food supplementation on song rate with a linear mixed model (LMM) using the R package "lme4". The model included male identity as a random effect and treatment (fed, control), nest stage (fertile, parental), and habitat (low quality, high quality) as fixed effects, and the treatment × nest stage and treatment × habitat interaction terms. We used a White-Huber corrected covariance matrix in the ANOVA to account for heteroscedasticity (White 1980), and partial sums of squares to account for unbalanced data (Fox 2008). We fit models using the restricted maximum likelihood (REML) method and used Wald chi-square values to examine the significance of variables and interaction terms. When interaction terms were not significant and did not improve model fit, we removed them from the final model. We examined habitat-specific differences in parental behaviour between fed birds and control birds with a Wilcoxon rank-sum test. Individuals included in the parental behaviour dataset were only sampled in one year.

We examined the effect of food supplementation and habitat quality on plasma androgens, baseline CORT concentrations, and male body condition with separate LMMs. We included individual as a random effect in each model because some males were represented more than once in the dataset. In the reduced models, we evaluated the effect of treatment as a fixed effect and the treatment × nest stage and treatment × habitat interaction terms. In the full models examining effects on hormones only, we also included year, age (SY, ASY), response latency, and bleed time as fixed effects and the treatment × year interaction term. We used the natural log of androgens and baseline CORT to improve normality of the model residuals. We corrected for heteroscedasticity and unbalanced data (as above), fit models using the REML method, and used Wald chi-square values to examine the significance of variables. We only included significant interaction terms in final models. Lastly, we examined the relationship between plasma androgens and baseline CORT concentrations with a linear model (LM), although we made no *a priori* prediction about the direction of this relationship.

We examined whether individual variation in plasma hormones was associated with male body condition and reproductive behaviours. We investigated the relationship between male body condition and plasma androgens and baseline CORT concentrations with LMs. We examined the association between male song rate and androgen and baseline CORT concentrations with LMMs; individual was included as a random effect because song rates of the same individual were often sampled during both the fertile and parental stages. We then examined the relationship between male parental effort (provisioning rate) and androgen and baseline CORT concentrations measured during the parental stage with LMs. We conducted paired-sample *t*-tests to compare differences between the fertile and parental stages in both androgen and baseline CORT concentrations (log-transformed) for 12 control males with hormone data from both nest stages.

Results

Effects of food supplementation and habitat quality

Male song rate was affected by supplemental feeding. Control males had significantly higher song rates than fed males, but this effect was seen only in low quality habitat (treatment × habitat, Wald $\chi^2_1 = 4.60$, $P = 0.03$; Fig. 2.2). Male song rate was significantly higher during the fertile stage than during the parental stage (LMM: Wald $\chi^2_1 = 37.69$, $P < 0.001$).

Food supplementation affected the parental behaviour of females, but not of males (Fig. 2.3). In low quality habitat, fed females provisioned at a significantly higher rate and delivered significantly more prey biomass than did control females (Fig. 2.3a, 2.3c). Fed females spent significantly more time at the nest than did control females in both low and high quality habitats (Fig. 2.3e).

Plasma androgen concentrations in males were affected by supplemental feeding and habitat quality. Contrary to our prediction, fed males had significantly lower androgen concentrations than control males during the fertile stage (Fig. 2.4a, Table 2.1: reduced model, although this effect only occurred in low quality habitat (Table 2.1: reduced model). We did not find a significant treatment effect on androgen concentrations, however, when we included the significant effect of response latency (Table 2.1: full model). Males in high quality habitat had significantly higher androgen concentrations than males in low quality habitat (Table 2.1:

reduced and full models).

Food supplementation affected baseline CORT concentrations and male body condition differently in the fertile and parental stages (Fig. 2.4b, 2.4c). As predicted, fed males had significantly lower CORT concentrations (Table 2.1: reduced and full models; Fig. 2.4b) and better-than-average body condition (LMM: Wald $\chi^2_1 = 4.87$, $P = 0.03$; Fig. 2.4c) relative to control males during their mate's fertile stage. However, and contrary to our prediction, fed males had significantly higher CORT concentrations during the parental stage than control males (Fig. 2.4b). We found no significant difference in CORT concentrations (Table 2.1) or body condition (LMM: Wald $\chi^2_1 = 3.54$, $P = 0.17$) between low and high quality habitat. Plasma androgen and baseline CORT concentrations were positively associated within males (LM: ß = 0.47 ± 0.13, $F_{1,216} = 12.7$, $P < 0.001$, $r = 0.24$).

Do plasma androgens regulate the mating-parental effort trade-off?

Androgen concentrations were associated with male body condition and male song rate, but not with male provisioning rate or male age. Males with high plasma androgens were in relatively poor body condition (LM: androgens: ß = -0.80 ± 0.20, $F_{1,216} = 14.1$, $P < 0.001$, $r = 0.26$) and had higher song rates (LMM: Wald $\chi^2_1 = 7.38$, $P < 0.01$) than males with low androgen concentrations. We found no relationship between androgen concentrations during the parental stage and male provisioning rate (LM: $F_{1,39} = 0.04$, $P = 0.84$). Plasma androgen concentrations did not differ between male age-classes (Table 2.1: full model).

As predicted, androgen concentrations were significantly higher in the fertile stage than in the parental stage (Table 2.1: full model), and males with higher androgen concentrations responded more rapidly to simulated territorial intrusions (Table 2.1: full model). Similarly, males that were sampled during both the fertile and parental stages had significantly higher androgen concentrations during the fertile stage (Paired *t*-test: $t_{11} = -3.03$, $P = 0.01$). However, we found substantial within-male variation in androgen concentrations, and 17% of the males in high quality habitat had higher androgen concentrations during the parental stage than during the fertile stage (Fig. 2.5).

Do baseline CORT concentrations indicate energetic stress, regulate parental effort?

Baseline CORT concentrations were associated with male body condition and mating effort, but not with male parental effort or age. Males with high baseline CORT concentrations were in lower-than-average body condition compared to males with low baseline CORT (LM: $ß = -0.26 \pm 0.10$, $F_{1,218} = 6.6$, $P = 0.01$, $r = 0.17$). In a post-hoc analysis, we found that song rate was also associated with energetic stress: baseline CORT concentrations during the parental stage were negatively associated with male song rate (LM: $ß = -0.15 \pm 0.08$, $F_{1,28} = 6.19$, $P = 0.02$). Male provisioning rate, in contrast, was not associated with CORT during the parental stage (LM: $ß = -0.16 \pm 0.26$, $F_{1,38} = 0.16$, $P = 0.69$). CORT concentrations also did not differ between male age-classes (Table 2.1: full model). As expected, baseline CORT concentrations increased with bleed time and exposure to STI (Table 2.1: full model). Baseline CORT was significantly higher in the fertile stage than in the parental stage (Table 2.1: full model), but males sampled during both the fertile and parental stages did not show a significant change in CORT concentrations across these stages (Paired *t*-test: $t_{10} = -0.82$, $P = 0.43$).

Discussion

We conducted this study to examine the degree to which behavioural plasticity of the birds can compensate for variation in resource conditions by adjusting their mating and parental effort to maximize individual reproductive success. In particular, we designed the food supplementation experiment to isolate the effects of food from all other factors that varied by elevation to determine the plasticity in behavioural and hormonal responses to differences in habitat quality.

Effects of food supplementation and habitat quality

Song rate was associated with higher androgen concentrations and likely reflected increased investment in extra-pair mate attraction. Supplemental feeding only affected male song rates and androgen concentrations in low quality habitat. However, fed males in low quality habitat sang at a lower, rather than higher, rate and had lower plasma androgen concentrations during their mates' fertile periods than did control males. Territories in low quality habitat were, on average, 30% larger than territories in high quality habitat (Kaiser, unpublished data). The reduced level of singing in low quality habitat indicated many sampling periods when no songs were detected.

Fed males on low quality territories may have spent more time away from their nest sites (where automated recorders were located) during the fertile stage to: 1) guard their social mates, 2) pursue extra-pair copulations (EPC), or 3) forage. Supporting the mate guarding explanation, females spend their time and energy foraging during the fertile stage to support egg production and only visit nest sites to lay eggs (Holmes et al. 2005). Males typically guard their social mates during the fertile period to prevent the loss of within-pair paternity (Chuang-Dobbs et al. 2001). Indeed, fed males in low quality habitat were less likely to be cuckolded (lose paternity) and also less likely to sire extra-pair young than control males (Chapter 3). Our song rate results suggest that supplemental feeding of males in low quality habitat may have allowed these males to increase their investment in mate guarding.

We also found that males breeding in high quality habitat had higher androgen concentrations than males in low quality habitat. Whether these habitat differences are due to nutrient availability (*e.g.,* Schoech *et al.* 2007), climatic conditions, or differences in social interactions among males remains unclear. Breeding density did increase with elevation at our site (mean [birds/10 ha] ± SE: low = 3.5 ± 0.3, high = 8.3 ± 0.5; 1995-2010). Higher densities in high quality habitat may promote greater competition among males for resources and EPCs, which could have affected androgen concentrations and the opportunity for extra-pair mating (Westneat and Sherman 1997, Horton et al. 2010).

Consistent with our predictions, fed males were better able to meet their energetic needs during the fertile stage relative to control males. Low baseline CORT concentrations were associated with low androgen concentrations. Considerable evidence has linked reduced body condition and reduced food availability to elevated baseline CORT, but less is known about the role of reproductive hormones in this context (reviewed in Schoech et al. 2009). CORT mobilizes glucose stores to meet energetic challenges, and baseline levels in breeding males are typically elevated to meet the energetic demands of T-regulated reproductive behaviour (Romero 2002). For this reason, CORT and T in birds can be positively correlated (e.g., van Hout et al. 2010). Elevated CORT may be adaptive to increase immediate availability of energy, but prolonged elevation due to poor weather and low resource availability may be maladaptive and interfere with reproduction (Wingfield 1985, Lynn et al. 2003, Schoech et al. 2007).

Fed females invested more into parental effort relative to controls, which likely allowed their social mates to spend more time on mate attraction and less on parental effort. Fed males had relatively high baseline CORT during their parental stage, and males with higher baseline

CORT sang at a higher rate. This suggests that males investing in extra-pair mate attraction via singing while also feeding nestlings were energetically stressed. Lozano and Lemon (1995) found a similar pattern with the congeneric yellow warbler (*S. petechia*); females with supplemental food visited their nests more frequently than control females, but the amount of parental care provided by males was unaffected by access to additional food. Higher baseline CORT in fed males also may reflect greater parental investment by males with increased resources. For example, male *Passer domesticus* (house sparrows) that invested more effort into reproduction had higher baseline CORT concentrations (Ouyang *et al.* 2011). Our results do not support this alternate hypothesis because we did not detect any effects of supplemental feeding on male parental effort.

In summary, food supplementation affected male and female reproductive effort, but the mechanism differed between the sexes and was generally more pronounced in low quality habitat. Fed females increased parental effort, whereas fed males appeared to increase mating effort instead. Two lines of evidence suggest that fed males were investing additional food resources into mating effort. First, fed males did not invest more into parental effort relative to controls. In particular, fed females increased their parental effort, which likely relieved their social mates from increased provisioning and provided more time and energy for these males to invest in mating behaviours. Second, fed males were in relatively poor body condition and had elevated baseline CORT concentrations during the parental stage, suggesting that they were not investing the supplemental energetic resources into self-maintenance behaviours.

Hormones and the regulation of male reproductive trade-offs

We found only indirect support for the role of plasma androgens in mediating male reproductive trade-offs between mating effort and parental effort. Most males had higher androgen concentrations during their social mate's fertile stage than during the parental stage. Male provisioning rate, but not song rate, was independent of androgen concentrations. Plasma androgens, therefore, did not appear to mediate the trade-off between extra-pair mate attraction and male parental effort in the black-throated blue warbler.

Seventeen percent of male black-throated blue warblers had higher androgen concentrations during the parental stage than during the fertile stage. This substantial within-male individual variation in hormone profiles may support different reproductive strategies. Although we do not have behavioural data from these males, individual variation in endogenous androgens were correlated with male feeding rates in several songbird studies (Pinxten et al. 2007; McGlothlin et al. 2007; Eikenaar et al. 2011).

Baseline CORT concentrations, while indicative of energetic stress in black-throated blue warblers, did not appear to regulate male parental effort. Male provisioning rates were independent of baseline CORT concentrations, although CORT was lower in the parental stage, when energetic demands on males are typically high, compared with the fertile stage. Other endocrine measures, such as acute stress-induced CORT concentrations, may be a better candidate for predicting parental effort (e.g., Ouyang et al. 2011). In particular, males that mount a stronger response to an acute stressor, as indicated by a greater change between baseline and post-stressed CORT concentrations, may be more likely to reduce their parental effort to invest more energy into regaining their homeostasis (Miller et al. 2009, Lendvai and Chastel 2010).

Conclusions

This study indicates that food availability and endocrine physiology interact to mediate reproductive trade-offs in a migratory songbird. We found that individuals invested less in reproductive effort and hormone production where food was most limited. Experimentally reducing food limitation for birds breeding under poor resource conditions increased reproductive effort for both males and females. Reproductive behaviours thus showed a plastic response to environmental conditions. Our results suggest that predicted shifts in black-throated blue warbler habitat quality due to climate change (Rodenhouse *et al.* 2008), could be mitigated by behavioural plasticity in mating and parental effort. The ability of black-throated blue warblers to compensate for future environmental change will depend in part on the cost of increased effort for adult survival. Further, our results indicate that plasma androgen is not the primary mediator of reproductive trade-offs in the black-throated blue warbler, nor does baseline CORT regulate parental effort, but that elevated circulating concentrations of both hormones are associated with reduced body condition. Manipulations of both food availability and hormone concentrations would be a powerful test of how reproductive trade-offs are mediated by interactions between the environment and the endocrine system.

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Table 2.1. Results from reduced and full linear mixed-models used to examine variation in plasma androgen and corticosterone concentrations of breeding male black-throated blue warblers, Hubbard Brook Experimental Forest, NH. Significant terms are in bold.

Model term[a,b]	**Coefficient**	**Wald χ^2**	***df***	***P***
Reduced model [Androgen] *n* = 225				
Intercept	1.12 ± 0.11			
Treatment (fed)	-0.75 ± 0.47	15.04	1	**< 0.001**
Treatment x Nest stage		83.64	2	**< 0.001**
Control x Parental stage	-1.40 ± 0.16			
Fed x Parental stage	-0.80 ± 0.45			
Treatment x Habitat		6.77	2	**0.034**
Control x Low quality	-0.45 ± 0.18			
Fed x Low quality	-0.28 ± 0.41			
Full model [Androgen] *n* = 186				
Intercept	1.12 ± 0.27			
Treatment (fed)	-0.16 ± 0.26	0.38	1	0.54
Nest stage (parental)	-1.20 ± 0.18	43.06	1	**< 0.001**
Habitat (low quality)	-0.36 ± 0.18	4.01	1	**0.045**
Year (2009)		3.67	3	0.30
2010	0.41 ± 0.23			
2011	0.29 ± 0.22			
2012	0.29 ± 0.25			
Age (ASY)	-0.31 ± 0.19	2.64	1	0.10
Response latency	-0.01 ± 0.00	6.04	1	**0.01**
Bleed time	0.03 ± 0.03	1.41	1	0.24
Reduced model [Corticosterone] *n* = 227				
Intercept	3.79 ± 0.06			
Treatment (fed)	-0.36 ± 0.28	0.35	1	0.56
Treatment x Nest stage		20.56	2	**< 0.001**
Control x Parental stage	-0.39 ± 0.09			
Fed x Parental stage	0.32 ± 0.27			
Treatment x Habitat		0.41	2	0.81
Control x Low quality	0.05 ± 0.11			
Fed x Low quality	-0.09 ± 0.25			
Full model [Corticosterone] *n* = 197				
Intercept	3.62 ± 0.14			
Treatment (fed)	-0.34 ± 0.21	0.06	1	0.81
Nest stage (parental)	-0.56 ± 0.10	27.96	1	**< 0.001**
Habitat (low quality)	0.03 ± 0.10	0.07	1	0.79
Year (2009)		22.18	3	**< 0.001**
2010	-0.43 ± 0.12			
2011	0.08 ± 0.12			
2012	-0.05 ± 0.13			

Age (ASY)	-0.05 ± 0.10	0.25	1	0.61
Response latency	0.01 ± 0.00	5.98	1	**0.014**
Bleed time	0.08 ± 0.01	30.39	1	**< 0.001**
Treatment x Nest stage	0.63 ± 0.27	5.51	1	**0.019**

[a] Each model included individual identity as a random effect.

[b] Full models have lower sample sizes than reduced models because of missing covariate data.

Fig. 2.1. Black-throated blue warbler (*Setophaga caerulescens*) female incubating in the Hubbard Brook Experimental Forest. Photo by Rebecca Koch.

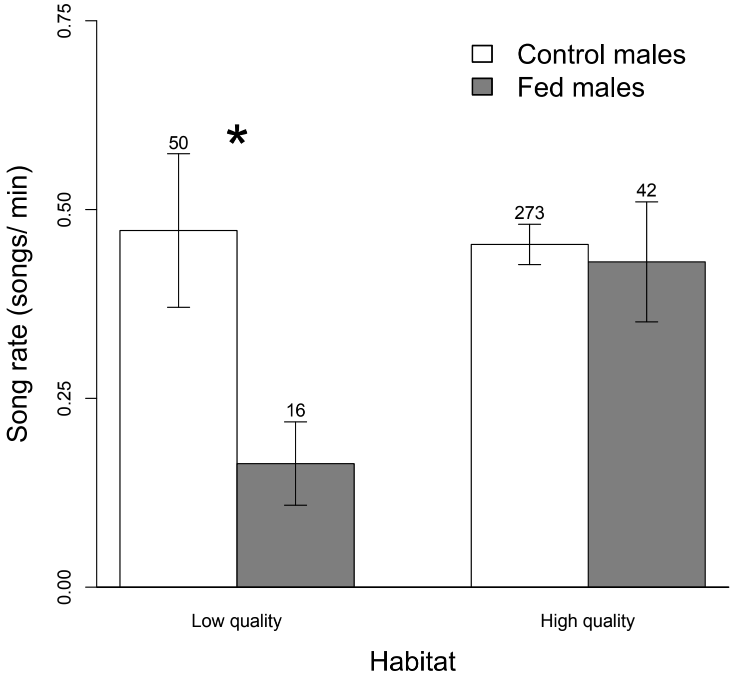
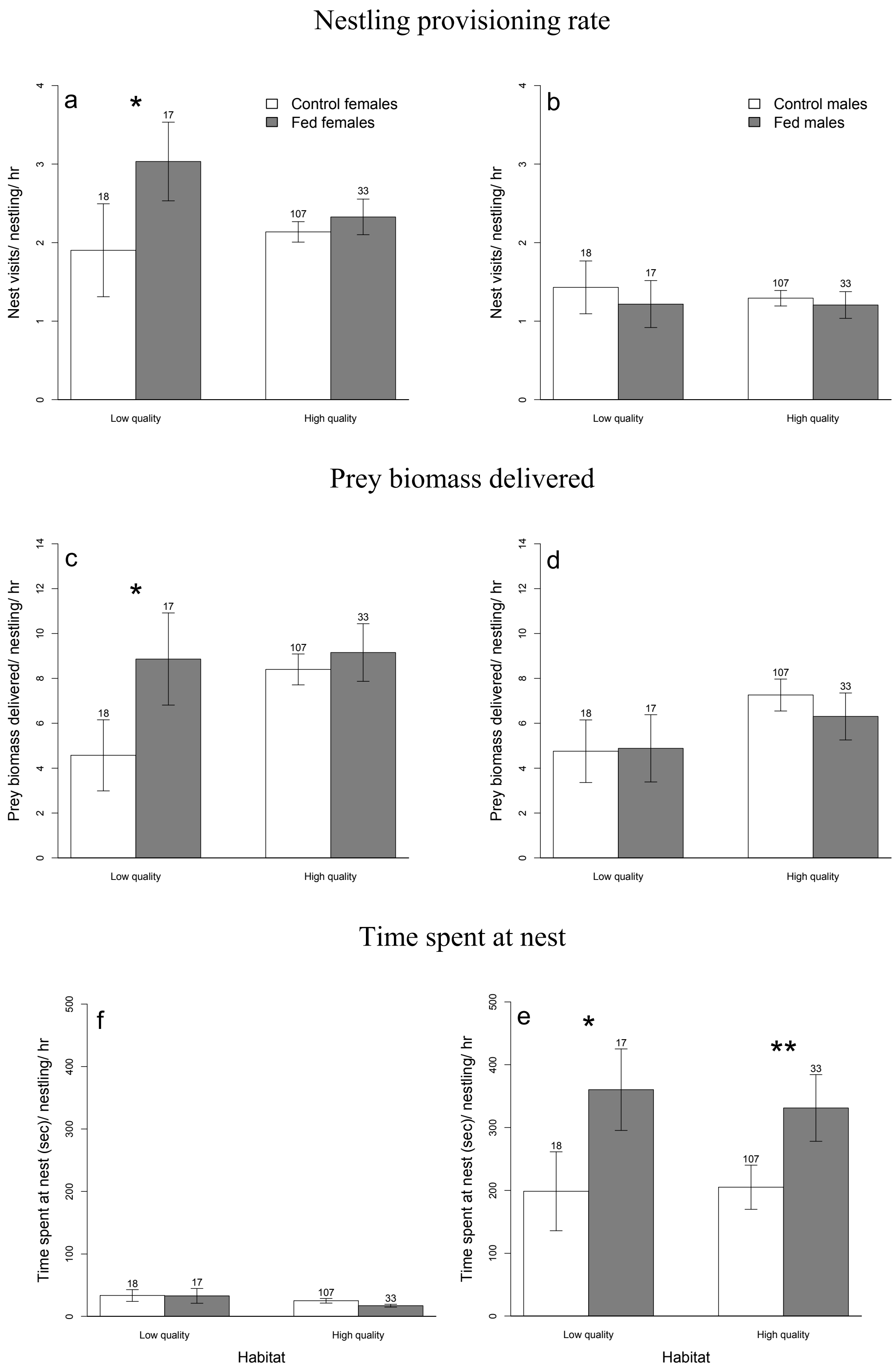


Fig. 2.2. The effect of food supplementation on male song rate by black-throated blue warblers breeding in low and high quality habitat. Data are pooled across nest stages and shown as means ± SE. Sample sizes are given above the error bars. Significance levels are from a linear mixed model controlling for fixed and random effects (**P* < 0.05).



Nestling provisioning rate

Prey biomass delivered

Time spent at nest

Fig. 2.3. The effect of food supplementation on a-b) nestling provisioning rate, c-d) prey biomass delivered to nestlings, and e-f) time spent at the nest by female (left panel) and male (right panel) black-throated blue warblers breeding in low and high quality habitat. Data are shown as means ± SE and sample sizes are given above the error bars. (*$P < 0.05$, ** $P < 0.01$)

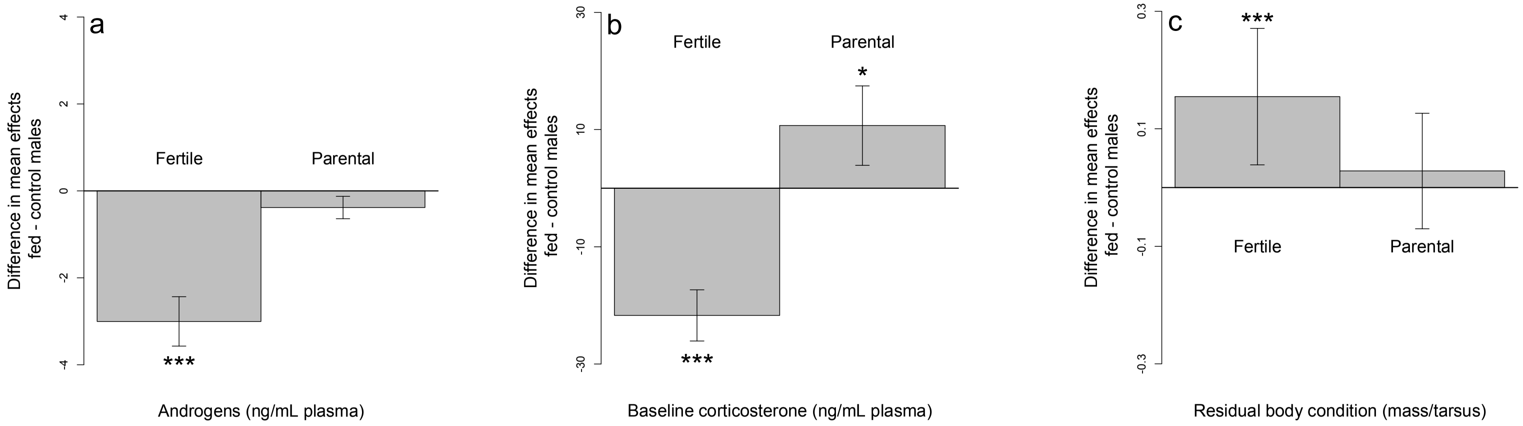


Fig. 2.4. Difference between fed and control males in a) plasma androgen concentrations, b) baseline corticosterone concentrations, and c) body condition by nest stage (fertile: fed = 8, control = 134; parental: fed = 19, control = 75). Bars show the difference in mean effects ± SE on fed males relative to control males. Significance levels are from linear mixed models (Table 2.1: reduced model) controlling for fixed and random effects (*$P < 0.05$, *** $P < 0.001$).

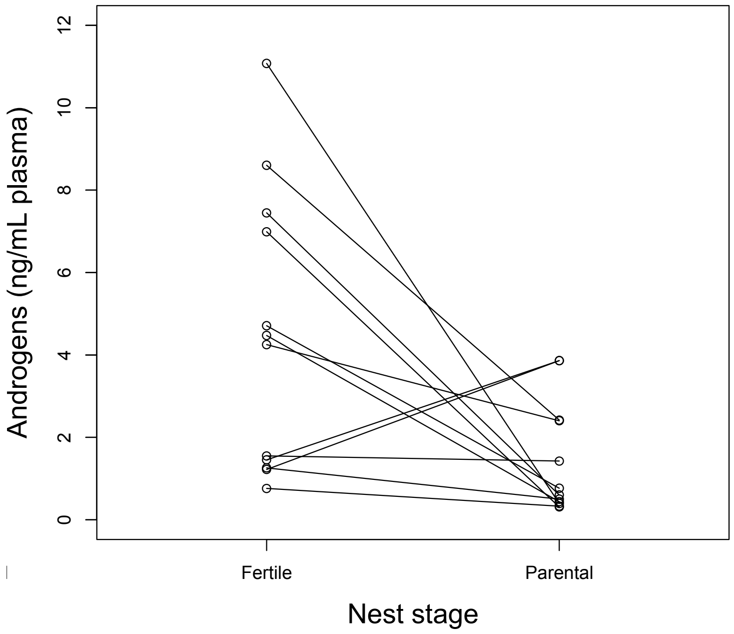


Fig. 2.5. Within-male variation in endogenous androgen concentrations for 12 male black-throated blue warblers from the control group sampled during both their social mate’s fertile stage and the parental stage in low and high quality habitat. Paired androgen concentrations of each male are connected by a solid line.

APPENDIX 2.1 Validation of method to detect black-throated blue warbler songs.

We generated song rates from dictaphone recordings using the band-limited energy detector in Raven 1.4 (BR Program 2004). To reduce the probability of false positives, we calibrated the detector by selecting ten recordings containing two counter-singing males. Individuals were identified using recordings of males that we targeted from 0530 – 1000 between May and July, hereafter "target-recordings", using a Marantz PMD 661 portable solid-state digital recorder at 48 kHz sampling rate, 24 bit-depth (D&M Professional, Itasca, Illinois), ME67 shotgun microphone capsules and K6 power modules (Sennheiser Electronic Corporation, Old Lyme, Connecticut). We adjusted the detector settings so that the focal male was detected and the neighbour male was excluded.

We randomly selected 50 recordings and determined the number of false positives and false negatives by visually inspecting the spectrograms. False positives and false negatives were relatively common (23% and 19% of total detections, respectively). False negatives were especially common in recordings with persistent background noise from rain or wind (82% of total detections). Therefore, if recordings contained background noise greater than 55 dB, we determined song rate by visually inspecting the spectrogram, which reduced the false negative rate to 8% of total detections. Additionally, we found that the occurrence of false positives increased to 30% of total detections when the recordings included songs of species that overlapped the frequency bandwidth of black-throated blue warblers (*e.g.* dark-eyed junco [*Junco hyemalis*] and winter wren [*Troglodytes hiemalis*]). Therefore, when recordings contained either of these two species, we excluded false positives by visually inspecting the spectrogram, which reduced the rate of false positives to <2% of total detections. The visual estimation of song rate did not differ significantly from the detector method (Paired *t*-test: $t_{28} = 0.29$, $P = 0.77$).

We should note that all recordings were initially inspected to examine the level of background noise and for the presence of species with overlapping frequencies to determine the method of analysis, visual or detector, for each recording. We discarded recordings with background noise exceeding 77 dB because it was not possible to distinguish the spectrogram of our study species from other bird species with overlapping frequencies at this level of background noise.

Occasionally, the detector still counted two songs in close succession, indicating two counter-singing males. For these recordings, we first attempted to identify the focal male using the target-recordings. If we did not have an exemplar of the focal male, we chose the male that sang consistently closer to the microphone in other recordings collected at the same nest. Importantly, in all cases in which we could identify the focal male using the target-recordings, it was also the male that sang consistently closer to the microphone. Thus, proximity to the microphone was a reliable method of identifying the focal male when recordings contained counter-singing males. We excluded from the analysis any recordings < 10 min due to battery failure.

CHAPTER 3

EXPERIMENTAL FOOD SUPPLEMENTATION REVEALS HABITAT-SPECIFIC REPRODUCTIVE INVESTMENT IN A MIGRATORY SONGBIRD*

*** S. A. Kaiser, T. S. Sillett, and M. S. Webster**

SUMMARY

Environmental constraints are hypothesized to affect the fitness costs and benefits associated with investment in extra-pair mating. Environmental factors that contribute to variability in extra-pair paternity among individuals within populations could influence genetic reproductive success. Habitat quality, such as food availability, may influence male investment in competing behaviors that affect paternity gains or losses. We used experimental food supplementation and natural variation in food availability to test the environmental constraints hypothesis in the migratory black-throated blue warbler (*Setophaga caerulescens*) breeding in low and high quality habitat. Food supplementation increased total annual reproductive success for males, but specific reproductive investment differed among habitats. Both food supplementation and naturally high food availability increased within-pair paternity. However, food supplementation in low quality habitat reduced extra-pair paternity and increased the probability of double-brooding. Our results support the hypothesis that environmental constraints affect individual variation in extra-pair paternity by influencing male reproductive trade-offs or female reproductive decisions. We suggest that males and females maximized their total annual reproductive success by adjusting their extra-pair mating behavior in response to food supplementation. Depending on habitat quality, these decisions either influenced opportunities for extra-pair mating or the likelihood of double-brooding.

1. INTRODUCTION

Genetic parentage analyses of socially monogamous birds have revealed that extra-pair paternity (EPP) is often an important component of male reproductive fitness (Webster et al. 1995, Griffith et al. 2002, Lebigre et al. 2012, Schlicht and Kempenaers 2013), and studies of intraspecific paternity patterns have found substantial spatial and temporal variation in EPP among individuals within populations (Canal et al. 2012). Yet, despite considerable research, the ecological factors driving these patterns in EPP within populations remain poorly understood (Petrie and Kempenaers 1998, Griffith et al. 2002, Westneat and Stewart 2003). Most studies examining factors influencing individual variation in mating effort have examined socio-ecological factors, such as local breeding synchrony and density, which may affect opportunities for males and females to encounter, and evaluate, available extra-pair mates (Stutchbury and Morton 1995, Westneat and Sherman 1997, Bennett and Owens 2002). Results from these studies, however, are inconsistent across species and populations (Westneat and Sherman 1997) and suggest that extra-pair mating behavior cannot be explained by a single ecological variable (Westneat and Stewart 2003, Westneat and Mays 2005). Other ecological factors that influence local breeding synchrony and density, such as food availability and climate, might also influence variation in EPP among individuals within populations, but these environmental variables have been far less explored.

Food availability and climate might influence extra-pair mating behavior by affecting the costs and benefits of pursuing extra-pair copulations (EPC). Unfavorable climatic conditions may reduce food availability and shift the allocation of limited resources from mating effort (*i.e.,* mate guarding, pursuit of EPC) to self-maintenance, and thereby affect the incidence of EPP

(Johnsen and Lifjeld 2003, Bouwman and Komdeur 2006). The few studies investigating the influence of food availability on individual mating effort, however, have mainly focused on female extra-pair mating behavior and within-pair paternity (WPP). For example, the predominant hypothesis predicts that females on low quality territories (*e.g.* with low food availability) should be less likely to cuckold their mate because they are energetically limited in their ability to circumvent guarding attempts by their social mates and/or unable to pay the energetic costs if males reduce parental care when cuckolded (constrained female hypothesis; Gowaty 1996). Empirical tests of this hypothesis have provided mixed support (Hoi-Leitner et al. 1999). Instead, the more common finding is that females on low quality territories are more likely to cuckold their mates (Westneat 1994, Václav et al. 2003, Rubenstein 2007, O'Brien and Dawson 2011). Suggested explanations for this pattern are that food availability may indirectly affect EPP if females on low quality territories necessarily forage further from their nest sites, possibly even on the territories of other males, increasing the probability of encountering extra-pair males (Westneat 1994, Václav et al. 2003, Rubenstein 2007). Alternatively, females may associate territory quality with aspects of male quality (*e.g.,* level of male parental care), such that males defending lower quality territories are more likely to be cuckolded (O'Brien and Dawson 2011).

Environmental factors can promote or constrain male extra-pair mating behavior. In particular, reduced energetic constraints from favorable climatic conditions and abundant food supplies may reduce the value of male parental care to the survival of nestlings and provide males with more time and energy to pursue EPC (Trivers 1972, Westneat et al. 1990). If favorable resource conditions permit multiple brooding, males face a trade-off between mate guarding to protect paternity in their second broods, providing parental care to fledglings from their first broods, and pursuing EPC (Westneat et al. 1990). The relative effort invested in each of these competing reproductive behaviors should be proportional to the expected gain in their reproductive success (Westneat et al. 1990). Examining reproductive success under different environmental contexts (*i.e.,* habitat quality) would help to disentangle the effects of environmental factors, such as food availability and climatic conditions, on both components of male reproductive success: WPP and EPP.

In this 4-yr study, we used a food supplementation experiment and natural variation in per-territory food availability to test the hypothesis that environmental constraints would increase individual variability in extra-pair paternity and reproductive success. We studied a population of the insectivorous, migratory black-throated blue warbler (*Setophaga caerulescens*). Habitat quality for this species increases from low to high elevations at our study site. At higher elevations, food availability is greater, nest predator abundance is lower, and the forest shrub layer used by the black-throated blue warbler for nesting is denser (Rodenhouse et al. 2003). First, we experimentally compared food-supplemented males to control males in low and high quality habitat (low and high elevations, respectively). We have previously shown that food-supplemented males ('fed males') invested more effort into mate guarding during their social mate's fertile stage and greater effort into extra-pair mate attraction during their parental stage than control males (Chapter 2). Food supplementation also increases the probability of double-brooding in our study population (Nagy and Holmes 2005a). Given these patterns, we tested the predictions that, relative to controls, fed males would have: 1) higher WPP (due to increased mate guarding), 2) higher EPP (due to increased effort to gain EPC), 3) higher incidence of double-brooding by their social mate, and 4) higher total annual reproductive success. Food availability is greatest at higher elevations, therefore, we predicted that food supplementation would more strongly affect these components of male reproductive success in low quality habitat. We expected little difference in the four fitness components between fed and control males in high quality habitat. We predicted that males on territories with naturally higher food availability during their social mate's fertile period would have higher WPP. Lastly, we examined the fitness consequences of differences in the relative effort invested by males in each component of reproductive success in low and high quality habitat.

2. METHODS

(a) *Study species, site, and field methods*

The black-throated blue warbler is a sexually dichromatic songbird that breeds in northern hardwood forests in eastern North America and migrates to the Greater Antilles for the non-breeding season (Holmes et al. 2005). We studied a breeding population of black-throated blue warblers from May through August 2009 – 2012 in the Hubbard Brook Experimental Forest, North Woodstock, New Hampshire, USA (43°56'N, 71°45'W). Males arrive to the breeding grounds in early May to establish territories and females arrive approximately one week later (Holmes et al. 2005). Pairs are socially monogamous with low levels of polygyny (5-10%) (Holmes et al. 1992) and relatively high levels of EPP (34%; 1995-1998) (Webster et al. 2001). Females build open-cup nests in the understory vegetation, primarily in hobblebush (*Viburnum alnifolium*) (Holmes et al. 2005). Females lay one egg per day (mean clutch size = 3.6, range = 2–5 eggs) (Holmes et al. 2005). Throughout the nest building and egg-laying stages, males guard their fertile mates (Chuang-Dobbs et al. 2001b). However, males do not participate in incubation and rarely feed their social mate at the nest during the 12-day incubation stage, but they do assist females in provisioning their nestlings (Holmes et al. 2005).

We collected data on three study plots at low (250-350 m; 85 ha), mid (450-600 m; 65 ha), and high (750-850 m; 35 ha) elevations. The study area is an intact northern hardwood forest dominated in the canopy by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*), with red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) increasing in abundance on the ridges (Schwarz et al. 2003). The dominant vegetation in the understory is hobblebush, striped maple (*A. pensylvanicum*), and saplings of canopy species.

In each breeding season, we marked all individuals and monitored nesting attempts on each study plot. We captured adults in mist-nets throughout the breeding season and marked each individual with an aluminum U.S. Geological Survey (USGS) leg band and a unique combination of three colored leg bands. At capture, we classified individuals as second-year (SY) or after second-year (ASY) using plumage characteristics (Pyle et al. 1997), and obtained *ca.* 70 µl of blood from the brachial vein. We collected *ca.* 30 µl of blood from the brachial vein of nestlings on day 6 (hatching = day 0) of the 9-day nestling stage and marked them with a USGS leg band. We stored blood in 500 µl of lysis buffer [0.1 Mm Tris (pH 8.0), 0.1 M EDTA, 10 mM NaCl, 0.5% SDS; 27] at 4°C until we conducted genetic analyses. We mapped the boundaries of male territories throughout the breeding season relative to the 50x50 m grid that delimited each study plot. We found nests by following females carrying nest material and adults carrying food. We monitored nests every other day throughout all nest stages and daily near hatch and fledge dates.

(b) *Food supplementation experiment*

Each breeding season after males were paired, we arbitrarily assigned 6 - 8 territories on each of the three study plots to the feeding treatment. We monitored 10 - 20 control territories per study plot, which were separated from fed territories by one or more territories. We began supplemental feeding first nest attempts 2 – 3 days after the onset of incubation and delivered food daily throughout all nest stages and at each nest. We established feeding trays 1 m from nests and initially delivered 5 g (37 kJ) of waxmoth larvae (Lepidoptera: *Galleria mellonella*). We moved the feeding trays to approximately 5 m from the nests once pairs were documented feeding and increased the amount of food delivered to 7 g (52 kJ). We provided 5 g of mealworms (Coleoptera: *Tenebrio monitor*) that we gut-loaded with cricket meal to increase protein and calcium content, and 2 g of waxmoth larvae. We delivered 14 g (104 kJ) of food during the nestling stage (10 g of mealworms and 4 g of waxmoth larvae) because adults regularly fed from feeding trays themselves and fed larvae to their young. Additional details on the design of the feeding tray and feeding experiment are described elsewhere (Chapter 2; Nagy and Holmes 2005). We successfully fed 11 pairs in 2009, 21 pairs in 2010, 18 pairs in 2011, and 18 pairs in 2012.

(c) *Natural food availability*

We estimated an index of food availability for each territory based on knowledge of the diets of adult and nestling black-throated blue warblers and their common foraging substrates. Black-throated blue warblers are insectivorous during the breeding season, feeding extensively on adult and larval Lepidoptera taken mostly from the surfaces of leaves in the understory and sub-canopy (Robinson and Holmes 1982, Holmes et al. 1986), and deliver mostly Lepidoptera larvae to their nestlings (Rodenhouse and Holmes 1992, Goodbred and Holmes 1996). The index of food availability per territory was a function of caterpillar biomass per leaf and the number of understory leaves per territory estimated following previously described procedures (Chapter 1). The index was based on visual caterpillar surveys in the shrub layer and understory leaf sampling (Holmes et al. 1979, Holmes and Schultz 1988, Sillett et al. 2004). To summarize, we counted and measured caterpillars (mm) on striped maple, and hobblebush leaves in the understory along plot-wide transect surveys conducted during four bi-weekly survey periods (1 June – 31 July). Caterpillar measures were converted to wet biomass (mg) using length-mass regressions (Rogers et al. 1977). Next, we estimated leaf abundances of each plant species on each territory with the Geospatial Modelling Environment (Beyer 2012) from kriged surfaces of leaf density derived from extensive understory leaf sampling (0-3 m height) on each study plot, and territory boundaries digitized in ArcGIS 10 (ESRI 2011). Territory size represented the area males defended over the entire breeding season. We totaled transect estimates of caterpillar biomass (mg), averaged per-transect estimates, and divided by 1000 leaves to obtain the mean caterpillar biomass per leaf for each plant species in each survey period. Then, we multiplied the two per-leaf quantities by leaf abundances within territories and summed each value. In the analysis examining the effects of natural food availability on WPP, we used the index of food availability from the survey period coinciding with the fertile stage. The effect of natural variation in food availability on the probability of a male siring extra-pair young is a part of a separate study, and is not considered here (Chapter 4).

(d) *Genetic analyses and paternity*

We extracted DNA from blood samples using Qiagen DNeasy blood and tissue kits (Qiagen, Valencia, CA). We genotyped adults and nestlings at six highly polymorphic microsatellite loci designed for *S. caerulescens* (*Dca28, Dca32*; Webster et al. 2001), *S. petechia* (*Dpu16, Dpu01*; Dawson et al. 1997)*, Vermivora chrysoptera* (*Vecr08*; Stenzler, Fraser, and Lovette 2004), and *Catharus ustulatus* (*Cuu04*; Gibbs et al. 1999). We amplified 1 μL of genomic DNA from each individual at each locus in a 10 μL polymerase chain reaction (PCR) containing 6.1 μL dH_2O, 1 μL 10X PCR buffer (Sigma), 1.2 μL 25 mM $MgCl_2$, 0.2 μL 10 mM deoxyribonucleotide triphosphates, 0.2 μL 10 μM forward (fluorescently labeled) and pigtail reverse primers, and 0.1 μL 2.5 U μL^{-1} Taq DNA polymerase (Invitrogen). We ran PCRs on a thermal cycler under the following conditions: 30 cycles of 94°C for 1 min, primer-specific annealing temperature for 1 min, 72°C for 45 s, and 72°C for 5 min. The PCR products were analyzed on an automated 3730 DNA Analyzer (ABI) and we scored alleles at each locus using Genemapper 3.7 (ABI).

We assigned paternity to offspring with known social mothers using CERVUS 3.0 (Kalinowski et al. 2007). We evaluated CERVUS assignments using likelihood scores and decision rules previously developed for this species (Smith et al. 2005). If the social father had a high likelihood score but mismatched the nestling at 1 or 2 loci, we investigated the possibility of null alleles or mistyping by repeating PCRs at mismatched loci and re-scoring the alleles. We accepted the CERVUS assignment of the social father as the genetic sire of a nestling if he mismatched at ≤1 locus. A nestling was considered sired by an extra-pair male if the social father was not listed as a potential candidate sire by CERVUS (due to having a negative LOD score) or mismatched the nestling at ≥2 loci. In these cases we identified the extra-pair sire as the male that mismatched at ≤1 locus and had the highest likelihood score. In some cases, no candidate males matched the nestling's genotype (*i.e.*, all had >2 mismatches with the nestling), and in these cases we considered the nestling to have been sired by an un-sampled male.

For each breeding male, we calculated three measures of annual reproductive success: within-pair paternity (number of young sired by males in their social mate's nests), extra-pair paternity (number of young sired by males in the nests of females that were not their social mate), and total reproductive success (total number of young sired by a male). We also determined the number of successful broods attempted by each breeding male's social mate (range = 0 – 2 broods).

(e) *Statistical analyses*

We examined the influence of food supplementation on WPP, EPP, and double-brooding in low and high quality habitats. Natural variation in food availability is similar, on average, between the mid and high elevations, relative to the low elevations (Rodenhouse et al. 2003). Thus, following methods in Chapter 2, we grouped data by habitat quality according to elevation (low quality = 250 – 350 m, high quality = 450 – 850 m). We examined the effect of supplemental feeding on 1) the probability of males maintaining WPP with a generalized linear mixed model (GLMM) and a binomial error distribution using the R extension package "lme4", 2) the probability of males gaining EPP with a GLMM and a binomial error distribution, 3) the probability of males' social mates fledging second broods with a GLMM and a binomial error distribution, and 4) total reproductive success with a GLMM and a Poisson error distribution. In these analyses, we included all candidate fathers in the dataset that had a social mate that attempted at least one nest and/ or that were assigned to at least one fledgling from the paternity analysis. In the GLMMs, we included treatment (fed, control), habitat (low quality, high quality), male age (SY, ASY), and treatment × habitat as fixed effects and included individual as a random effect because some males bred more than once in a season and/ or in more than one year of the study. We did not include year in the models because of low sample sizes of fed males in each year. All analyses were performed using R version 2.15.3 (R Core Development Team 2013). We present results as untransformed means ± SE.

We examined the relationship between territory quality (index of food availability during the social females' fertile stage) and WPP (nests with or without extra-pair young) with a GLMM and a binomial error distribution. We log-transformed the food index data to meet model assumptions. We excluded fed nests to examine the influence of natural variation in per-territory food availability. In the model, we included individual as a random effect and the index of food availability, year, date of clutch completion, habitat (low quality, high quality), and male age as fixed effects.

Lastly, we investigated the contribution of WPP, EPP, and double-brooding to total reproductive success with a GLMM and a Poisson distribution. We specified individual as a random effect in the model, and WPP, EPP, and double-brooding (yes or no), and all pairwise interactions as fixed effects. In this analysis, we combined data from control and fed males because results were similar when we ran separate analyses by treatment (results given in Appendix 3.1).

3. RESULTS

(a) *Patterns of paternity*

We genotyped 452 adult males, 332 adult females, and 943 nestlings to determine parentage of 286 broods of black-throated blue warblers (Table 3.1). We assigned paternity to 860 (91.1%) nestlings, 529 (61.5%) of which were sired by their social father, and 331 (39.2%) of which were assigned to an extra-pair sire. The combined probability of paternal exclusion for all loci was >99.9%. The remaining 83 offspring were not assigned to any sire, but were considered to be extra-pair young because they mismatched their social father. Thus, our paternity analyses showed that 414 (43.8%) offspring were extra-pair young and 155 nests (54.2%) contained at least one nestling sired by an extra-pair male. Extra-pair paternity rates were similar across the four years of this study (Table 3.1). Males gained between 0 – 8 extra-pair young in a breeding season. Older (ASY) males had higher total annual reproductive success than yearling (SY) males in both low and high quality habitat across treatments (Table 3.2). Older males, relative to yearling males, were significantly more likely to gain EPP and fledge second broods, but WPP did not differ significantly between male age-classes (Table 3.2).

(b) *Food supplementation, habitat quality and reproductive success*

Fed males had significantly higher reproductive success than control males (Table 3.2), but the effects of food supplementation on male reproductive investment differed between low and high quality habitat (Table 3.2, Figure 3.1). In both habitats, food supplementation significantly increased the probability of males maintaining WPP (Table 3.2, Figure 3.1). In low quality but not in high quality habitat, food supplementation both significantly reduced the probability of males gaining EPP and increased the probability of a male's social mate fledging a second brood (Table 3.2, Figure 3.1). The overall probability of gaining EPP did not differ significantly between low and high quality habitat (Table 3.2). The total effect of food supplementation was to significantly increase the total annual reproductive success for males in both low and high quality habitat (Table 3.2), which ranged from 0 – 13 genetic young among breeding males.

(c) *Natural food availability and paternity*

Natural variation in territory quality (excluding fed territories) also resulted in individual differences in WPP. Males on territories with high food availability during their social mate's fertile stage were more likely to maintain WPP (GLMM: $\chi_1^2 = 4.67$, $P = 0.03$, n = 225 nests). The probability of a male maintaining WPP was not influenced by clutch completion date (GLMM: $\chi_1^2 = 1.15$, $P = 0.28$), and did not differ significantly between low and high quality habitat (GLMM: $\chi_1^2 = 0.06$, $P = 0.81$), among years (GLMM: $\chi_3^2 = 0.49$, $P = 0.92$), or between male age-classes (GLMM: $\chi_1^2 = 0.06$, $P = 0.81$).

(d) *Fitness gains with male reproductive investment*

Total annual reproductive success was greater for males that maintained WPP (GLMM: $F_{1,275} = 14.09$, $P < 0.001$), gained EPP (GLMM: $F_{1,275} = 11.61$, $P < 0.001$), and both maintained WPP while gaining EPP (WPP × EPP: GLMM: $F_{1,275} = -5.80$, $P < 0.001$) (Figure 3.2a). Double-brooding increased total annual reproductive success, but only when males also maintained WPP (double-brooding: GLMM: $F_{1,275} = 1.10$, $P = 0.27$; WPP × double-brooding: GLMM: $F_{1,275} = -1.97$, $P = 0.048$) (Figure 3.2a). Relative effort invested by males in WPP, EPP, and double-brooding in response to food supplementation in low and high quality habitat (Figure 3.1) contributed to differences in the overall gains in reproductive success (Figure 3.2b).

4. DISCUSSION

We examined the fitness consequences of individual variation in male reproductive investment by black-throated blue warblers in response to experimental food supplementation and natural variation in food availability. Food supplementation increased male reproductive success in both low and high quality habitats, but specific patterns of reproductive investment differed across habitats (Figure 3.1). Food supplementation and naturally high food availability resulted in high levels of WPP, regardless of habitat. However, food supplementation in low quality habitat reduced EPP and increased double-brooding, whereas fed and control males did not differ in these fitness measures in high quality habitat. The results of this study provide support for the hypothesis that environmental constraints can strongly affect individual variation in extra-pair paternity by either influencing male reproductive trade-offs or female reproductive decisions. We discuss these potential behavioral mechanisms and the adaptive significance of male and female adjustments in extra-pair mating behavior in response to food supplementation and habitat quality.

Potential behavioral mechanisms

Although we do not know if EPC in black-throated blue warblers are male- or female initiated, males were often observed singing on the edges of their territories or intruding on neighboring males' territories when nearby females were fertile (authors' pers. obs.). Females likely assess these intruding males and might actively solicit EPC from males while remaining on their territories. Thus, we assume that both male and female black-throated blue warblers have the potential to control EPC, and that food supplementation could have affected both female and male extra-pair mating behavior.

We did not find support for the constrained female hypothesis, which predicts that females on low quality territories are less likely to solicit EPC (resulting in higher WPP) because they have more to lose if their social mate withdraws parental care (Gowaty 1996). Black-throated Blue Warbler males on both food-supplemented territories and those with naturally high food availability were less likely to be cuckolded (high WPP), independent of habitat type. These results are consistent with the few avian studies that have experimentally increased food availability in species where females actively seek and solicit EPC (Westneat 1994, Václav et al. 2003, O'Brien and Dawson 2011). One potential benefit of female faithfulness could be increased parental care by males (Houston and McNamara 2002). We have correlative evidence that older male black-throated blue warblers reduce parental care when cuckolded (Chuang-Dobbs et al. 2001a). However, food supplementation did not influence male parental effort and fed females had higher parental effort than control females (Chapter 2). These results indicate that the primary benefit of food supplementation to females was direct access to additional resources. Therefore, we suggest that fed females were less likely to cuckold their social mates because supplemental feeding improved the perceived quality of their social mate while increasing the true quality of their territory.

Experimentally reducing energetic constraints through supplemental feeding influenced male reproductive trade-offs mediated by habitat quality and affected paternity outcomes. Fed males in low quality habitat were more likely to maintain WPP and had a higher likelihood of double-brooding than control males. Contrary to our prediction, fed males were less likely to gain EPP than control males. These results suggest that, instead of pursuing EPC, fed males in low quality habitat invested additional resources into mate guarding to reduce cuckoldry (Chuang-Dobbs et al. 2001b). The most pronounced effect of food supplementation in low quality habitat, however, was to increase the likelihood of double-brooding (see also Nagy and Holmes 2005). Males of multiple brooding species often provide substantial parental care to fledglings once their social mate begins building a second nest (Evans Ogden and Stutchbury 1997). This could reduce the time and energy available for males to pursue EPC (Westneat

1990). The paternity patterns we document in low quality habitat suggest reproductive trade-offs between mate guarding, provisioning fledglings from first broods, and pursuing EPC. As predicted, the probability of gaining EPP or double-brooding did not differ between fed and control males in high quality habitat. However, fed males in both high and low quality habitat had higher WPP.

The paternity outcomes in each habitat were consistent with our previous behavioral study, which found that fed males invested more effort into mate guarding during their social mates' fertile stage (Chapter 2). However, variation in EPP may occur via different mechanisms resulting from male and female extra-pair mating behavior. Fed males in both habitats may have invested additional resources into foraging for their own self-maintenance rather than seeking EPC, but we do not have the behavioral data to test this possibility. The WPP patterns we observed in both habitats also could have been driven by female reproductive decisions based on their perceptions of male and/ or territory quality. Hypotheses that focus on one sex remain inadequate in explaining this behavioral variation (Westneat and Mays 2005).

Fitness-maximizing male reproductive investment?

Habitat-specific reproductive investment in WPP, EPP, and double-brooding in response to food supplementation resulted in higher male reproductive success for fed males. Interestingly, fed males and males on territories with naturally higher food availability in both habitats had higher WPP than control males. In species that double-brood, WPP may contribute more to total reproductive success than EPP (e.g., Westneat 1990). The variance in reproductive success in double-brooded species is often higher due to differences in the probability of double-brooding, rather than EPP (Dolan et al. 2007). Behavioral mechanisms may also differ between single-brooded and double-brooded species, because males that do not double-brood may have more time to seek EPC (Dolan et al. 2007). The habitat-specific patterns we observed in relative effort invested in EPP and double-brooding by fed and control males likely resulted from differences in both the opportunity for EPC and for double-brooding. Breeding density was lower in low quality habitat (Chapter 2), thus the encounter rate among potential extra-pair mates and the opportunity for EPC was likely lower than in high quality habitat. We also know from previous work that the probability of double-brooding increases with food availability (Nagy and Holmes 2005a). We suggest that the higher reproductive success of fed males in low quality habitat resulted from their investment of additional resources into: 1) mate guarding to assure WPP where the opportunity for EPP was low, and 2) double-brooding when food supplementation increased the probability of fledging a second brood. Male and female black-throated blue warblers maximized their total annual reproductive success by adjusting their extra-pair mating behavior in response to food supplementation. Depending on habitat quality, these decisions either influenced opportunities for extra-pair mating or the likelihood of double-brooding (Westneat and Sherman 1997, Nagy and Holmes 2005a, Stewart et al. 2010, Ryder et al. 2012).

5. CONCLUSIONS

We have shown with both experimental and correlational data that, in a migratory songbird with relatively high levels of EPP, environmental constraints can strongly affect total annual reproductive success of males by influencing WPP and EPP, as well as the likelihood of double-brooding. Previous work on our breeding population of black-throated blue warblers has shown that double-brooding is associated with higher annual fecundity (Nagy and Holmes 2005a, 2005b, Townsend et al. 2013). Our results extend these conclusions by experimentally demonstrating strong effects of habitat quality on both the likelihood of double-brooding and securing higher genetic reproductive success via WPP. Habitat quality mediated the effects of food supplementation on investment in extra-pair mating and explained much of the variation in EPP among individuals within the population. An understanding of the spatial and temporal variation in individual patterns of EPP will be important to determine under what environmental and social contexts an individual is likely to invest in securing WPP over gaining EPP to maximize their total genetic reproductive success.

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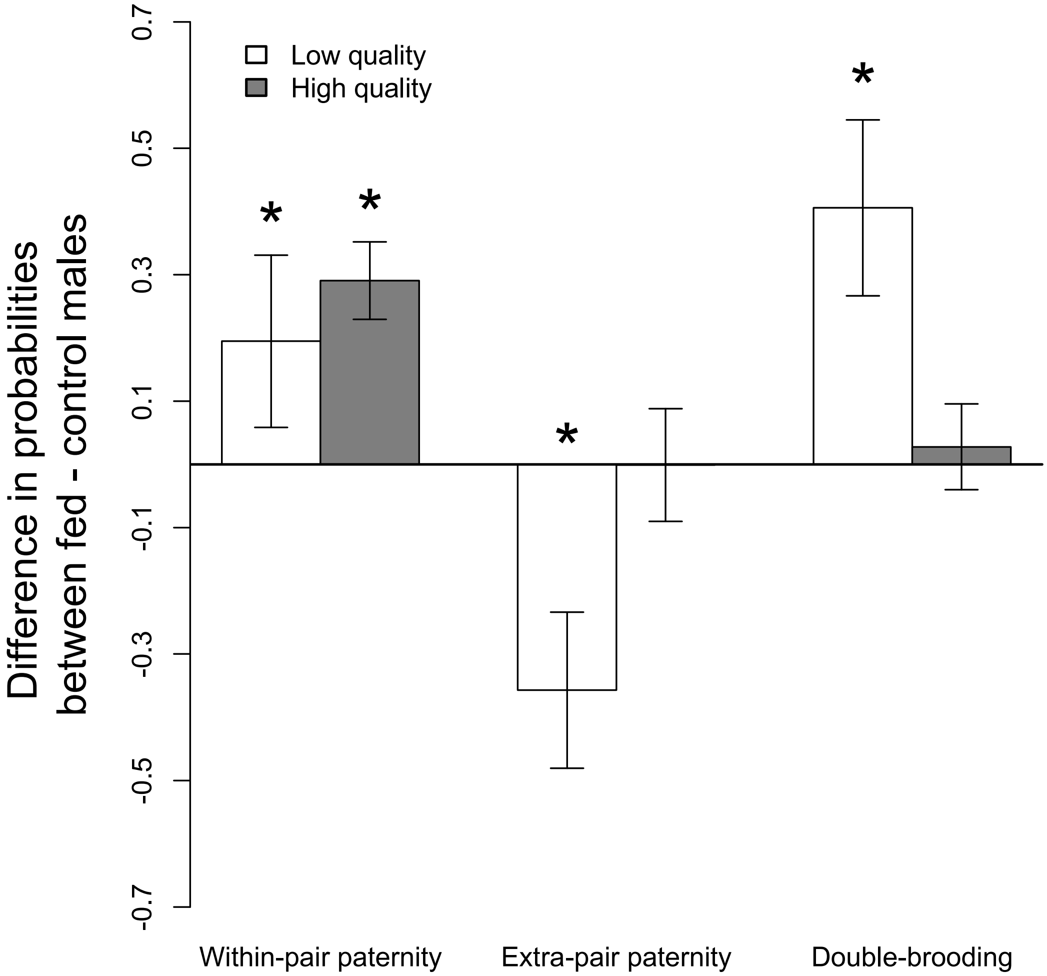

Figure 3.1. Difference between fed and control male black-throated blue warblers in the probability of securing within-pair paternity, gaining extra-pair paternity, and fledging second broods (double-brooding) by habitat quality. Bars show the difference ± SE in the probabilities of fed males relative to control males (*n* = males; low quality: fed = 14, control = 44; high quality: fed = 39, control = 178). Significance (* $P < 0.05$) was assessed with GLMMs after controlling for age effects (Table 3.1). Analyses included all candidate fathers that had a social mate that attempted at least one nest and/or that were assigned to at least one fledgling from the paternity analysis.

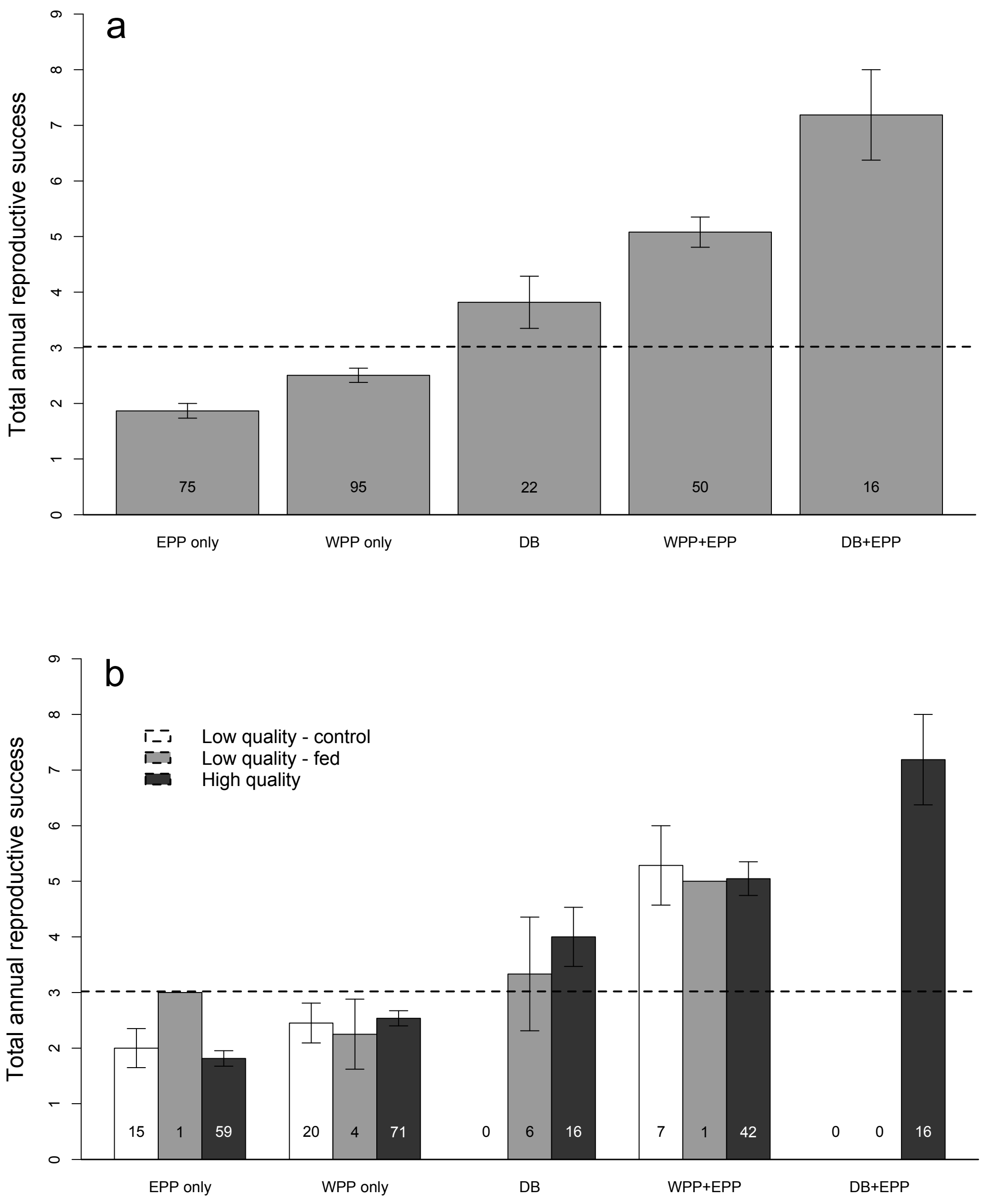

Figure 3.2. Relative contribution of within-pair paternity (WPP), extra-pair paternity (EPP), and double-brooding (DB) on total annual reproductive success of male black-throated blue warblers for a) all males and b) males by treatment and habitat (data are pooled across treatments in high quality habitat). Categories include males that were: single-brooded, lost WPP and gained EPP (EPP only), single-brooded and maintained WPP (WPP only), double-brooded and maintained WPP (DB), single-brooded, maintained WPP and gained EPP (WPP+EPP), and double-brooded, maintained WPP and gained EPP (DB+EPP). The dashed line indicates the mean total annual reproductive success, 2009-2012. Samples sizes are given at the base of the bars

Table 3.1. Extra-pair paternity rates of black-throated blue warblers at the Hubbard Brook Experimental Forest, NH across years.

Year	**% EPY (*n*)**	**% Broods with EPY (*n*)**
2009	41.7 (163)	53.8 (52)
2010	41.8 (189)	55.6 (63)
2011	43.1 (274)	55.8 (77)
2012	46.7 (321)	57.4 (94)
Total	43.8 (947)	54.2 (286)

EPY: extra-pair young

Table 3.2. Fixed effects from GLMMs* examining the effects of food supplementation on the probability of males maintaining paternity in their own nest (within-pair paternity), gaining paternity in other females' nests (extra-pair paternity), having social mates that fledged second broods (double-brooding), and total reproductive success in black-throated blue warblers. Significant terms are in bold.

	Within-pair paternity			**Extra-pair paternity**			**Double-brooding**			**Total reproductive success**		
Model term *n* = 275	β ± SE	*F*	*P*	β ± SE	*F*	*P*	β ± SE	*F*	*P*	β ± SE	*F*	*P*
Treatment (Fed)	1.85 ± 0.59	3.13	**<0.01**	-0.06 ± 0.36	-0.18	0.86	0.14 ± 0.47	0.29	0.77	0.23 ± 0.12	16.14	**0.05**
Male age (SY)	-0.16 ± 0.37	1.26	0.21	-0.02 ± 0.35	-2.49	**0.01**	-0.74 ± 0.37	-2.03	**0.04**	-0.13 ± 0.12	-2.14	**0.03**
Habitat quality (Low)	0.36 ± 0.28	-0.43	0.67	-0.64 ± 0.26	-0.06	0.96	-1.88 ± 1.04	-1.81	0.07	-0.18 ± 0.09	-1.01	0.31
Treatment x Habitat quality	-0.84 ± 0.96	-0.87	0.38	-1.73 ± 0.90	-1.93	**0.05**	3.41 ± 1.25	2.74	**<0.01**	-0.17 ± 0.25	-0.68	0.50

* Each model included individual male as a random effect

APPENDIX 3.1

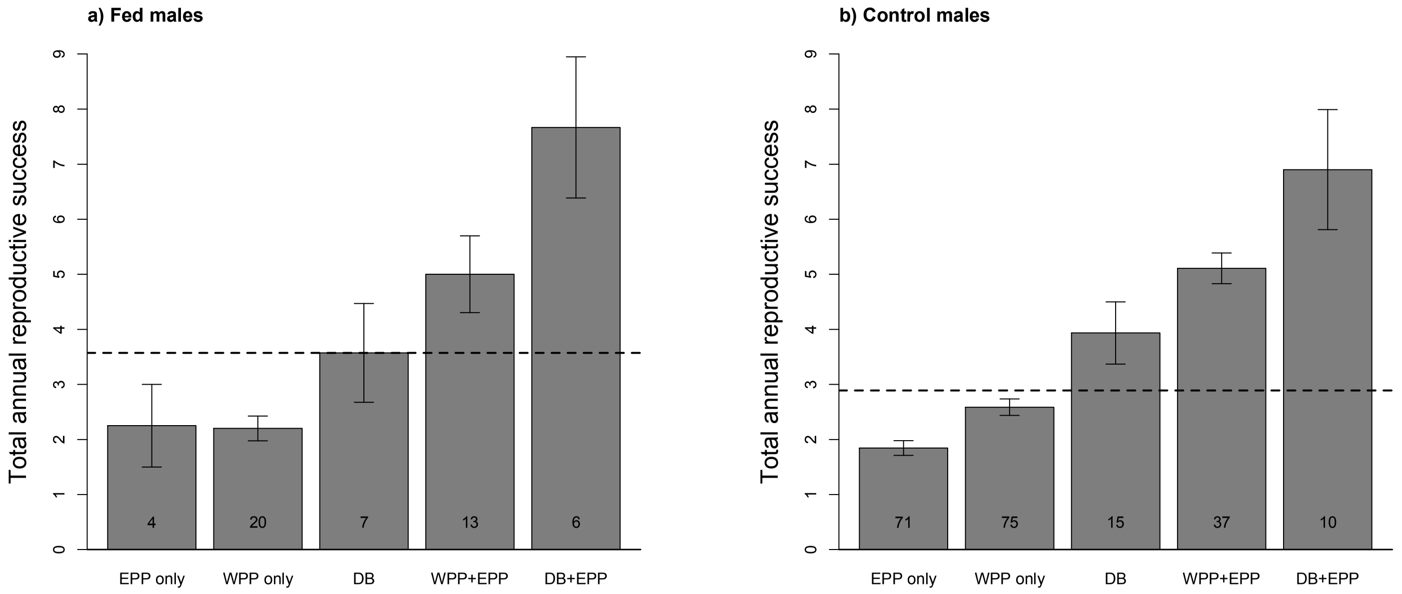

Figure S3.1. Relative contribution of within-pair paternity (WPP), extra-pair paternity (EPP), and double-brooding (DB) on total annual reproductive success of a) fed and b) control male black-throated blue warblers. Categories include males that were: single-brooded, lost WPP and gained EPP (EPP only), single-brooded and maintained WPP (WPP only), double-brooded and maintained WPP (DB), single-brooded, maintained WPP and gained EPP (WPP+EPP), and double-brooded, maintained WPP and gained EPP (DB+EPP). The dashed line indicates the mean total annual reproductive success, 2009-2012. Data are pooled across habitats and samples sizes are given at the base of the bars.

CHAPTER 4

ECOLOGICAL AND TEMPORAL CONSTRAINTS AFFECT THE OPPORTUNITY FOR EXTRA-PAIR MATING IN A MIGRATORY SONGBIRD*

S. A. Kaiser, B.B. Risk, T. S. Sillett, and M. S. Webster

ABSTRACT

Extra-pair paternity (EPP) is highly variable within populations of socially monogamous birds, but the ecological factors that drive mating decisions and patterns of EPP remain poorly understood. The spatial and temporal distribution of available mates and resources may influence individual variation in the probability of gaining EPP. We hypothesized that reproductive trade-offs and resource availability within males' territories would influence where and when males sired extra-pair young (EPY). We studied the Black-throated Blue Warbler (*Setophaga caerulescens*), a migratory songbird with bi-parental care, at the Hubbard Brook Experimental Forest, NH, USA. We found that extra-pair mating opportunities were not limited to neighbors' nests and that males minimized the constraints imposed by reproductive trade-offs by primarily siring EPY during the incubation stage of their social mates. Males with high quality territories had a lower probability of siring EPY than males with low quality territories, and also were less likely to sire EPY in nests far away from their territories. We detected no effect of local breeding density on the distance between females and their extra-pair mates. These results indicate that factors affecting the relative timing of breeding between a male's social mate and available fertile females may have a strong effect on the opportunity for extra-pair mating. Reproductive trade-offs appear to constrain male extra-pair behavior in black-throated blue warblers, rather than spatial factors.

INTRODUCTION

The spatial and temporal distribution of potential mates and resources necessary for reproduction should strongly influence mating systems (Emlen & Oring 1977), including patterns of extra-pair mating (Westneat et al. 1990). Accordingly, the spatial and temporal scales of interactions among individuals can potentially influence variance in mating success and the strength of sexual selection (Westneat & Mays 2005; Canal et al. 2012; Taff et al. 2013). For example, if females are spatially limited to choosing among neighboring males and males are limited to assessing the receptivity of neighboring females, variance in male mating success would be low and the strength of selection would be weak even when mating preferences exist (Webster et al. 1995, 2001). In contrast, if mating opportunities are not limited by distance, then individuals can fully express mating preferences, which would lead to greater variance in male mating success and stronger sexual selection. An examination of individual variation in these relationships between distance and the probability of siring EPY could provide insight into the spatial processes that constrain extra-pair mating success (Johnsen & Lifjeld 2003; Westneat & Mays 2005; Canal et al. 2012). Likewise, examining male variation in the timing of extra-pair fertilizations (EPF) in relation to the their social female's fertile and parental stages would elucidate temporal constraints on extra-pair mating success due to reproductive trade-offs (e.g., Evans et al. 2008; Canal et al. 2012). To explain individual variation in extra-pair paternity (EPP) within a population and how socio-ecological factors increase both the opportunity for extra-pair mating and the strength of sexual selection, we need to understand the scale at which a male's probability of gaining EPP is affected by both their spatial and temporal proximity to fertile females.

The spatial distribution of breeding individuals influences the variation in EPP rates within populations (reviewed in Westneat & Sherman 1997), as high local breeding density can increase the frequency of encounters among males and females and promote extra-pair copulations (EPC) (Westneat et al. 1990; Møller & Birkhead 1993). Yet, within-species comparisons have provided mixed support for this pattern (Westneat & Sherman 1997; Bennett

& Owens 2002; Griffith et al. 2002), perhaps, due to differences among studies in the scale used to examine breeding density effects (Webster et al. 2001; Westneat & Stewart 2003; Westneat & Mays 2005; Stewart et al. 2010). A common assumption in parentage studies is that males are spatially limited to siring EPY in their neighbors' nests (e.g., Yezerinac et al. 1995; Freeman-Gallant et al. 2005). The number of territories between extra-pair males and cuckolded males is sometimes used as a criteria for accepting or rejecting parentage assignments (Sardell et al. 2010). Some passerine studies, however, have found that a significant proportion of EPP result from interactions among females and non-neighboring males (Dunn et al. 1994; Westneat & Mays 2005; Dolan et al. 2007; Canal et al. 2012). These results suggest that breeding density might affect individual variation in the distance traveled to pursue EPC (Taff et al. 2013).

Males are assumed to be temporally limited to seek EPC because of reproductive trade-offs (Westneat et al. 1990; Magrath & Komdeur 2003). Males must trade-off the fitness costs and benefits of pursuing EPC with guarding their social mates from intruding extra-pair males (Westneat et al. 1990). This trade-off arises because males are unable to simultaneously perform behaviors aimed at gaining additional matings and investing in parental care (*e.g.,* nest defense, provisioning) (reviewed in Stiver and Alonzo 2009). Knowing when males are more likely to sire EPY can reveal how potential trade-offs influence optimal reproductive investment (e.g., Westneat 1988; Canal et al. 2012). High levels of synchrony among breeding females could lead to lower rates of EPP due to mate guarding or parental constraints (Birkhead & Biggins 1987; Westneat et al. 1990). Local breeding density and synchrony can also interact to influence EPP rates (Thusius et al. 2001; Stewart et al. 2010), which indicates the importance of taking into account both the proximity of potential extra-pair males to fertile females, as well as their social mate's reproductive stage.

Ecological factors, such as food availability, that structure the spatial and temporal distribution of potential mates, also affect the probability of EPP (Westneat et al. 1990; Westneat & Stewart 2003; Westneat & Mays 2005; Rubenstein 2007). When food is abundant the relative importance of male parental care may be lower (Dunn & Robertson 1992, Chapter 1). Males can allocate more time and energy to search for fertile females (Magrath & Komdeur 2003). Extra-pair males with abundant food on their territories might also be able to travel further distances to pursue EPC due to reduced energetic and temporal constraints.

Here, we examine the influence of reproductive trade-offs on the spatial and temporal distribution of EPP within a population of Black-throated Blue Warblers (*Setophaga caerulescens*). This songbird is sexually dichromatic and socially monogamous (Holmes et al. 2005), but EPP is common (54.2%; Chapter 3). We hypothesized that males would seek EPC with females nesting in close proximity to their territories to minimize the costs (*e.g.,* cuckoldry, reduced parental care) of searching for extra-pair females, and that the distance traveled for EPC would be correlated with local breeding density. We predicted that the probability of a male siring EPY would: 1) decrease with distance between fertile females and candidate males, 2) decrease with increasing local breeding density (due to increased male-male competition for EPC), and 3) increase the distance travelled to pursue EPC for males nesting in areas of low local breeding density relative to males nesting in areas of high local breeding density (because males would need to cross fewer intervening territories). We also hypothesized that territory quality would influence spatial patterns of EPP, due to the energetic cost associated with seeking EPC. We predicted that the distance between extra-pair males and the nests at which they sire EPY would be positively associated with ecological factors indicative of territory quality (caterpillar density and abundance), and that the probability of a male siring EPY would decrease with distance less rapidly as territory quality increased. Finally, we hypothesized that males would trade-off pursuing EPC with investment in mate guarding to reduce cuckoldry, and with parental care to increase fledging success. We predicted that males would be more likely to sire EPY during their social mate's incubation stage compared to their social mates' fertile periods or when both sexes were provisioning offspring.

METHODS

Study population

We studied Black-throated Blue Warblers over four breeding seasons (2009-2012) at the 3160-ha Hubbard Brook Experimental Forest, New Hampshire, USA (43°56'N, 71°45'W). The study area encompassed three study plots at low (250-350 m; 85 ha), mid (450-600 m; 65 ha), and high (750-850 m; 35 ha) elevations along a 600-m elevation gradient. Black-throated Blue Warblers were common throughout our study area, but their density increased with elevation (Rodenhouse et al. 2003). The dominant canopy species across the elevation gradient were sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*); red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) were more abundant on the high elevation plot (Schwarz et al. 2003). Hobblebush (*Viburnum alnifolium*), the preferred nest substrate for the warblers, along with striped maple (*A. pensylvanicum*) and beech saplings, were the dominant shrub-layer plants (Schwarz et al. 2003, Holmes et al. 2005). The biomass of caterpillars, which warblers feed on extensively (Robinson & Holmes 1982; Holmes et al. 1986), was correlated with the density of deciduous leaves in the understory and increased from low to high elevations (Rodenhouse et al. 2003).

The breeding ecology of Black-throated Blue Warblers has been studied at Hubbard

Brook since the mid-1980s (Holmes 2007, 2010). Males and females arrive in early May (Holmes et al. 2005). Males defend 1-4 ha territories, with the smallest territories in areas where the shrub layer is dense (Steele 1992; Holmes et al. 2005). All males held territories and we had no evidence of floaters (Marra & Holmes 1997; Sillett et al. 2004). Females build open-cup nests in the shrub layer (Holmes et al. 2005), lay one egg per day (mean clutch size = 3.6 eggs), and begin incubation when the penultimate egg is laid (Holmes et al. 2005). Females sometimes attempt second broods after fledging first broods, depending on resource conditions (Nagy & Holmes 2005). Males guard their mates during nest building and egg-laying, attend their nests infrequently while their social mate is incubating (*ca.* 12 days), and provide parental care to their nestlings (*ca.* 10 days) (Holmes et al. 2005).

Field methods

We marked birds, mapped territories, and monitored nest attempts on each study plot. We caught adult birds in mist-nets and marked each adult with a unique combination of three colored leg bands and an aluminum U.S. Geological Survey (USGS) leg band. Young were marked on day 7 of the nestling stage with a USGS leg band. We aged adults at capture as second-year (SY) or after second-year (ASY) using plumage characteristics (Pyle et al. 1997). For paternity analysis, we collected *ca.* 70 µl of blood from the brachial vein of adults and *ca.* 30 µl of blood from the brachial vein of nestlings. We stored blood in lysis buffer [0.1 Mm Tris (pH 8.0), 0.1 M EDTA, 10 mM NaCl, 0.5% SDS; 27] at 4°C until genetic analyses were conducted. We mapped the boundaries of male territories every few days throughout the breeding season relative to the 50x50 m grid that delimited each plot. Males were observed for 15-20 min periods and their movements, singing locations, and agonistic interactions with neighboring males were recorded

(Holmes et al. 1992; Sillett et al. 2004). We located nests by following females carrying nest material or by following adults carrying food, and monitored nests every other day throughout all nest stages and daily near hatch and fledge dates. Nests were usually discovered during nest building.

We sampled leaf abundance and caterpillar biomass on each study plot to assess habitat quality. We conducted leaf sampling in the understory (0-3 m height) to estimate a leaf abundance index for hobblebush and striped maple leaves in the shrub layer. We conducted biweekly visual caterpillar surveys from June 1 – July 31 along plot-wide transect surveys to estimate caterpillar biomass (mg) per leaf for each foraging substrate. Field procedures are described in detail elsewhere (Holmes et al. 1979; Holmes & Schultz 1988; Sillett et al. 2004).

Genetic analysis of parentage

We used six highly polymorphic microsatellite loci to determine the paternity of 748 nestlings from 257 broods (141 nestlings from 51 broods in 2009, 145 from 55 in 2010, 207 from 65 in 2011, and 255 from 86 in 2012) and the genotypes of 368 males and 226 females across all four years. We excluded individuals that were part of a food supplementation study conducted during these same 4 years including pairs for which either the social male or extra-pair sire was fed during the female's fertile stage. The microsatellite loci were isolated from the genome of *S. caerulescens* (*Dca28, Dca32*; Webster et al. 2001), *S. petechia* (*Dpu16, Dpu01*; Dawson et al. 1997)*, Vermivora chrysoptera* (*Vecr08*; Stenzler et al. 2004), and *Catharus ustulatus* (*Cuu04*; (Gibbs et al. 1999). We isolated genomic DNA from red blood cells using Qiagen DNeasy blood and tissue extraction kits (Qiagen, Valencia, CA) and amplified 1 μL of genomic DNA from each individual at each locus in a 10 μL polymerase chain reaction (PCR). The master mix for the PCR contained 6.1 μL dH_2O, 1 μL 10X PCR buffer (Sigma), 1.2 μL 25 mM $MgCl_2$, 0.2 μL 10 mM deoxyribonucleotide triphosphates, 0.2 μL 10 μM forward (fluorescently labeled) and pigtail reverse primers, and 0.1 μL 2.5 U μL^{-1} Taq DNA polymerase (Invitrogen). PCR were run on a thermal cycler for 30 cycles beginning with denaturation at 94°C for 1 min, primer-specific annealing temperature (50°C, 52°C, or 54°C) for 1 min, extension at 72°C for 45 s, followed by 72°C for 5 min. We analyzed the PCR products with a size reference (GeneScan-500 LIZ®) on an automated 3730 DNA Analyzer (ABI) and we scored alleles at each locus using Genemapper 3.7 (ABI).

We ran paternity analyses for each study plot and breeding season separately and included males sampled within and adjacent to the plot boundaries as candidate sires. We assigned paternity with the assumption that the social mother was the genetic mother (Webster et al. 2001) using CERVUS 3.0 (Kalinowski et al. 2007), which we later verified by examining the frequency of mother-offspring mismatches. We evaluated CERVUS assignments of genetic fathers using trio log likelihood scores (LOD), that are calculated to statistically distinguish among non-excluded candidate sires and taking into account the genotypes of the known mother and potential mistyping errors. We repeated PCRs at mismatched loci if the social father had a high likelihood score to determine if mismatches were likely due to genotyping error and if individuals appeared to be homozygous at a locus because of allelic dropout or a null allele. We accepted CERVUS assignments if the candidate sire mismatched offspring at ≤1 locus. However, when two males had similar LOD scores and both had only 1 mismatch, we also evaluated the CERVUS assignment by considering if the candidate sire was the social father or sired offspring in the same nest, or if the mismatch appeared to result from a genotyping error or null allele. If the social father was excluded as a potential candidate sire by CERVUS (because of a negative

LOD score) or mismatched offspring at ≥2 loci, we considered the nestling to have been sired by an extra-pair male. If no candidate males matched the nestling's genotype, we considered the nestling to have been sired by an un-sampled extra-pair male (8.8% of cases).

Prior to exclusion of nestlings and broods associated with fed birds, we assigned paternity to 860 of 943 (91.1%) nestlings and we identified the extra-pair sire of 331 (39.2%) nestlings. We could not identify the extra-pair sire of 83 offspring that mismatched their social father (combined probability of paternal exclusion for all loci = 0.999). Thus, 414 (43.8%) nestlings were EPY and 155 nests (54.2%) contained at least one EPY. Details of within-pair and extra-pair paternity rates across years of this study are provided in (Chapter 3).

Spatial and temporal patterns in EPP

We estimated all pairwise distances between each fertile female and candidate male. We considered the nest site to approximate the location of a fertile female and the centroid of a territory to represent the location of a candidate male. We used a Global Positioning System (GPS) to obtain geographic coordinates of nests on the study plot. Territory boundaries were georeferenced to the 50x50 m plot grid points; territory polygons were digitized; and the geographic coordinates of centroids and size (m^2) of each territory were extracted using ArcGIS 10 (ESRI 2011). We calculated the Euclidean distance between each nest (female locations) and each territory centroid (candidate male locations, excluding social mates) in the same plot and year.

We calculated an index of breeding density for each candidate male by taking the inverse of the average pairwise Euclidean distances between the territory centroids of all males that fell within a buffer. The buffer was a circle centered at each candidate male's territory centroid and with an area equal to three times the average territory size (1.55 ha) of all males in the population (i.e., a circle with radius equal to 210 m). If no male other than the focal male fell within the buffer, then we set the breeding density index to zero; this occurred for only one male.

We determined territory quality for each male as an index of food (caterpillar density), which was calculated from the estimated density of caterpillars on suitable food substrates (*i.e.,* leaves) and the density of food substrates on the male's territory (Robinson & Holmes 1982; Holmes et al. 1986). We estimated hobblebush and striped maple leaf abundance indices for each territory from kriged surfaces of leaf abundance summed over all raster cells within territory boundaries using ArcGIS 10 and the Geospatial Modelling Environment (Beyer 2012) following previously described analytic methods (Chapter 1). Then, for each fertile female/candidate male pairing, we calculated a caterpillar density index as {(hobblebush abundance) × (mg leps / hobblebush leaf) + (striped maple abundance) × (mg leps / striped maple leaf)} / (sum of hobblebush and striped maple abundance), where (mg leps / hobblebush leaf) and (mg leps / striped-maple leaf) were equal to the mean from the survey period coinciding with the fertile stage of the female.

We examined the timing of EPF relative to the clutch completion date of the extra-pair sire's social mate. Studies have indicated that in species with high rates of EPP, the risk of cuckoldry begins several days prior to egg-laying and extends over most of the egg-laying stage, but that EPC is more likely to result in successful fertilizations near the clutch completion date (Birkhead & Møller 1992). Accordingly, we followed Chuang-Dobbs et al. (2001b) and defined a female's fertile period to include the three days prior to clutch initiation through the day the penultimate egg was laid. For a subset of males with reproductive data for all their known social mates (n = 292), we calculated the difference in the clutch completion dates (Δ CCD) between the fertile female's nest and the extra-pair sire's social female's nest. Positive values indicate males that fertilized eggs with extra-pair females after the completion of his social female's clutch, and negative values indicate EPF that occurred prior to the completion of his social female's clutch. We also created an indicator variable, "Sign of Δ CCD", that = 1 if the extra-pair sire's social female completed her clutch prior to the fertile female and = 0 otherwise. Including this indicator variable allowed for flexibility in modeling the functional form of the relationship between Δ CCD and the probability of EPY, rather than assuming a monotonic relationship. We determined which nest stage a male sired EPY by relating the Δ CCD to the onset and length of each nest stage of his social mate (*i.e.,* fertile, incubation, nestling, post-fledging).

Statistical analyses

We created three generalized linear mixed models (GLMMs) to examine the factors affecting *P*(EPY), the probability of a candidate male siring EPY with a fertile female. All models had a binomial error distribution and logit link function. The response variable = 1 if the candidate male sired EPY with the fertile female and = 0 otherwise. Male and nest identities were included as random effects. Male identity accounted for repeated observations of each candidate male in the fertile female/candidate male pairings and nest identity accounted for repeated observations of the fertile female in the fertile female/candidate male pairings. We also included distance, male age, and their interaction in all models because these variables were important predictors of *P*(EPY); see Results. All analyses were conducted in R 3.0.1 with the lme4 package (Bates et al. 2013).

In Model 1, we examined how *P*(EPY) was associated with distance between male territories and the nests of fertile females and conspecific density. *P*(EPY) was modeled as a function of the distance between the nest and the centroid of the male's territory, distance^2, distance^3, the candidate male's index of breeding density, and age-class of both the candidate male and the social male of the fertile female (both defined as SY = 1, ASY = 0). Distance and breeding density were standardized to have sample mean equal to zero and sample variance equal to one; polynomial terms were a function of standardized distance. The following interactions were also included: distance × breeding density; distance × candidate male age; distance × social male age; and candidate male age × social male age.

Model 2 considered the correlations between candidate males' territory quality and *P*(EPY). Note that breeding density and caterpillar density were correlated ($R = 0.19$), which partly motivated our use of different models. *P*(EPY) was a function of distance, territory size, caterpillar density within a candidate male's territory, candidate male age, and the following interactions: distance × territory size; distance × caterpillar density; territory size × caterpillar density (*i.e.,* index of caterpillar abundance within a territory); distance × candidate male age; distance × territory size × caterpillar density. Distance, territory size, and caterpillar density were standardized as above.

Our third model assessed the effect of breeding synchrony on *P*(EPY). This analysis was conducted on the subset of fertile female/candidate male pairings for which full reproductive data were available (see "Breeding synchrony index" above). *P*(EPY) was a function of Δ CCD, sign of Δ CCD, distance, extra-pair sire age, and the following interactions: Δ CCD × sign of Δ CCD; sign of Δ CCD × distance; Δ CCD × distance; distance × extra-pair sire age. Distance was standardized as above.

We also modeled the factors affecting the distance between extra-pair males and the nests at which they sired EPY. We restricted this fourth analysis to the fertile female/candidate male pairs in which the candidate male successfully sired EPY. We used a linear mixed model in which the distance between a nest and the centroid of the extra-pair sire's territory was the response variable. Territory size, caterpillar density, and the interaction between the two were model predictors. We log-transformed the distance variable to normalize the residuals, and included candidate male as a random effect. *P*-values were estimated using lmerTest (Kuznetsova et al. 2013)

RESULTS

The probability of a male siring EPY was significantly greater with females that nested in close proximity to their territories, but was not significantly correlated with breeding density or the age of social males (Table 4.1). Older males generally had a higher *P*(EPY), but the probability declined rapidly with distance (Table 4.1, Figure 4.1). The effect of distance decreased more slowly for SY than ASY males. *P*(EPY) approached 0 near 500 m for both male age classes. After 500 m, the probability increased somewhat for SY males but not ASY males. The proportion of males that sired EPY >500 m was significantly greater for SY males (Chi-square test: $\chi^2_1 = 10.75$, $P = 0.001$; SY: $n = 19/50$ territories; ASY: $n = 7/66$ territories).

The probability of a male siring EPY decreased with caterpillar density estimated on candidate males' territories, but was not significantly associated with territory size (Table 4.2). We found a significant negative interaction between distance and caterpillar density (Table 4.2), indicating that the probability of siring EPY declined more rapidly with distance in high quality territories than in low quality territories, which was contrary to our prediction.

For males that sired EPY, the clutch completion date for extra-pair females was on average 6.2 days later than the clutch completion date of their social females (SE = 1.02, n = 109, paired t-test: P<0.0001; Figure 4.2). Males whose social females completed their clutch prior to the extra-pair female had a significantly higher probability of siring EPY (Table 4.3), and the probability of siring EPY decreased as Δ CCD increased (Table 4.3).

Some, but not all measures of territory quality influenced the distance between fertile females and their extra-pair mates. As territory size increased, this distance significantly increased ($F_{1,120} = 1.02$, $P = 0.03$). The distance between fertile females and extra-pair sires was not significantly affected by caterpillar density within a territory ($F_{1,120} = 4.62$, $P = 0.31$). Instead, males on territories with high caterpillar abundance were significantly more likely to sire EPY in nests that were in close proximity to their territories (territory size × caterpillar density $F_{1,120} = 4.70$, $P = 0.03$), which was contrary to our prediction.

DISCUSSION

Extra-pair mating opportunities are limited by a host of factors, including the proximity of fertile females to potential extra-pair males and/ or by reproductive trade-offs that affect the pursuit of EPC, and environmental conditions that structure the distribution of potential mates in both space and time should affect patterns of EPP (Westneat et al. 1990; Magrath & Komdeur 2003; Westneat & Mays 2005). The majority of studies examining these spatial and temporal relationships have focused on how breeding density and synchrony influence the overall frequency of EPP within a population and have found mixed results (Griffith et al. 2002). Here, we focused on how spatial and temporal factors influenced individual variation across males in the probability of siring EPY. The variance in genetic reproductive success among individuals determines the strength of sexual selection on male traits that increase mating success (Arnold &

Wade 1984; Webster et al. 1995), and this variance may be driven by EPP in socially monogamous systems (Webster et al. 2007). Our aim was to identify the ecological sources of variation that might contribute to differential reproductive variance among males via EPP, regardless of male phenotype.

We predicted that the probability of a male siring EPY would increase with proximity to fertile females because of the reduced cost of searching for EPC, but decrease with increasing local breeding density because of greater competition among potential extra-pair males. We found that local breeding density had no effect on this probability (see also Chuang et al. 1999), but that distance strongly predicted whether a male would sire EPY. However, many EPY were sired by males quite distant from the fertile female, as only 26% of EPY were sired by males located ≤140 m from the focal nest (the average diameter of a male's territory) and 45% of EPY were sired by males within 210 m (the distance that we used as a buffer to estimate local breeding density within an area three times the average territory size). These results differ from a previous study of this population that suggested that the majority of EPY were sired by neighboring males (Webster et al. 2001). The spatial resolution of that earlier study, however, was limited because the number of territories between extra-pair males and cuckolded males was used to assess distance, which ignored the variability in male territory sizes and the unoccupied habitat between territories. Yet, territory size has since been shown to confound analyses examining spatial distributions of EPP patterns (Westneat & Mays 2005, this study). That previous study also was conducted in the high elevation zone where breeding densities are at the high end of the range, which may have underestimated individual variation in the spatial patterns of EPP. We observed substantial variation in the distance between fertile females and the territories of extra-pair sires when we examined spatial patterns across the elevation gradient, with some extra-pair sires having territories over a kilometer away from the nests where they sired EPY.

Local breeding density was not associated with the distance between females and extra-pair sires. We predicted that individuals nesting in low densities would necessarily travel further to encounter extra-pair mates than individuals nesting in high densities. Similar to our findings, Taff et al. (2013) found no difference in the distance between fertile females and extra-pair sires nesting at different densities in the Common Yellowthroat (*Geothlypis trichas*). The lack of an effect of breeding density on distance traveled to seek EPC may be explained by differences in the opportunity for EPP. When the opportunities for EPP are lower, as in areas of low local breeding densities (Westneat & Sherman 1997; Møller & Ninni 1998; Griffith et al. 2002), the pay-off to males for increasing the search distance to find available fertile females should also be low. Theoretically, a male breeding in an area of low density should gain more from investing in behaviors that reduce cuckoldry rather than pursue EPC (Westneat et al. 1990).

Contrary to our predictions, we found that males with high quality territories were less likely, rather than more likely, to sire EPY compared to males with low quality territories, and also were less likely to sire EPY in nests farther away from their territories. When we examined the effect of territory quality on the subset of males that did sire EPY, we found that the distance between fertile females and extra-pair sires was also greater for males with low quality territories than males with high quality territories. This result is surprising because males on high quality territories should have reduced foraging demands leaving more time and energy to search for fertile females, and possibly over a larger area. For example, we previously showed that males on food supplemented territories and territories with naturally high food availability were less likely to be cuckolded (lose paternity) (Chapter 3). However, we were unable to distinguish between the alternative behavioral explanations that males with higher quality territories invested additional resources into mate guarding to reduce cuckoldry or that territory quality influenced female mate choice based on the perception of mate and/ or territory quality.

A third, but related possibility to these hypotheses is that territory quality may influence the foraging movements of individuals involved in extra-pair mating interactions (Gray 1997). That is, if males and females on low quality territories have to travel further distances to forage, then these males would be predicted to sire EPY in nests further away from their territories than males that remain within or closer to their territories to forage (Rubenstein 2007). Females would be predicted to have a greater opportunity to seek EPC outside of their social mates' territory leading to greater cuckoldry (Václav et al. 2003; Rubenstein 2007). Birds on low quality territories have been found to forage further away from their nest sites than individuals on high quality territories (Rubenstein 2007), and pairs provided supplemental food spend more time near their nest sites (Westneat 1994). At Hubbard Brook, food-supplemented males breeding in low quality habitat were less likely to sire EPY, suggesting that these males were spending more time on their territories (Chapter 3). If pairs on low quality territories had to travel further from their territories to forage, this may also explain why, at >1 km, yearling males had a higher probability of siring EPY than older males. Generally, older males arrive earlier to the breeding grounds than yearling males and preemptively occupy high quality breeding sites (Holmes et al. 1996). Although not all yearling males are relegated to low quality territories, we suggest that yearling males may have had to travel further distances to forage resulting in the higher proportion of yearling males that sired EPY >500 m from their territories.

Males may experience trade-offs between the benefits from engaging in extra-pair behavior and the costs from reduced mate guarding or parental care (Westneat et al. 1990). In our study, the majority of Black-throated Blue Warbler males were able to avoid reproductive trade-offs by siring EPY after the their social mates began incubating their clutch, but before their eggs hatched. Males sired EPY an average of 6.2 days into their social mates' 12-13 day incubation periods. These patterns suggest that males maximize paternity gains by first assuring their paternity in their own clutch by guarding their social mate during late nest-building and egg-laying, pursue EPC while their female is incubating, and then invest in parental care once their chicks hatch. In a similar study of the Pied Flycatcher (*Ficedula hypoleuca*), males also sired most EPY during their social mates' incubation stage (Canal et al. 2012). Although, a higher proportion of males in their study also obtained EPF while their social mate was laying eggs compared to our results. Timing of EPF was also sensitive to whether a male's social mate had completed her clutch before the extra-pair female's date of clutch completion rather than after this date. Males paired to females that initiate nesting earlier in the breeding season thus may not experience the constraints of mate guarding, and be able to increase their *P*(EPY) in nests initiated by females later in the breeding season. A few studies of migratory songbirds have demonstrated a fitness advantage for males that initiate breeding early (Møller 2003; Reudink et al. 2009; Cooper et al. 2011).

Although the majority of males sired EPY during their social mates' incubation stage, 21% of males sired EPY while they were still feeding nestlings and/ or fledglings. Male Black-throated Blue Warblers actively participate in parental care activities and increase their rate of provisioning as their nestlings approach fledging (Holmes et al. 2005). Brood division of fledglings is common, but males provide the majority of parental care to fledglings from first broods if their social mate attempts a second brood, and provide little parental care to fledglings from second broods (Holmes et al. 2005). Males should invest more into pursuing EPC during the parental stage when the number of receptive neighboring females increases (Chuang et al. 1999; Chuang-Dobbs et al. 2001a). However, the number of receptive female neighbors is typically low when males are feeding nestlings and fledglings (Chuang-Dobbs et al. 2001a). This could explain the low proportion of EPF that occur during the parental stage. Our results imply that while energetic or temporal constraints on males can lead to trade-offs between mating effort and parental effort, some males appear to take advantage of additional mating opportunities during periods of parental care.

In summary, we have shown in the Black-throated Blue Warbler that extra-pair mating opportunities were not entirely limited to local neighborhoods and that males engaged in extra-pair behavior most often after their social mates began incubation but before their young hatched. Males on high quality territories where breeding densities were generally greater, were less likely to sire EPY, although we found no evidence that breeding density facilitated or constrained extra-pair mating interactions. Interestingly, yearling males and males on low quality territories sired EPY further away from their territories than older males or males on high quality territories, which suggests that food availability influenced the foraging movements and spatial distribution of EPP. Although EPP has been shown to increase variation among males in reproductive success, we agree with the conclusion of Webster et al. (2001) that the strength of sexual selection may be weak because EPP occurred at a relatively small (<500 m) spatial scale. We suggest that factors affecting the timing of breeding may have a stronger effect on the opportunity for extra-pair mating (and also sexual selection) when reproductive trade-offs constrain male extra-pair behavior. In particular, arriving early may be important for males to pair with high quality females, secure paternity with their social mate, and then invest in behaviors to attract extra-pair mates (Spottiswoode et al. 2006; Reudink et al. 2009). Studies that examine relationships between the timing of arrival and the variance in EPP would be valuable to understand the factors that promote sexual selection in migratory birds.

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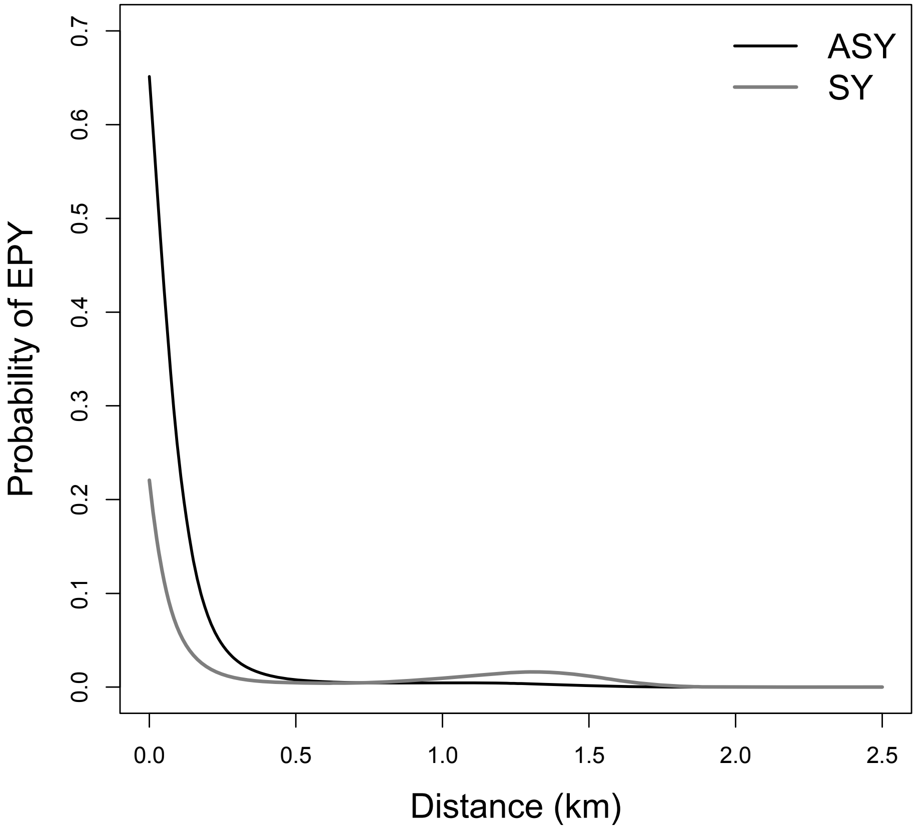

Figure 4.1. The effect of distance between nests (female locations) and the centroids of candidate males' territories on the probability of a male siring extra-pair young (EPY) by male age-class (ASY = after-second year, SY = second-year) in the Black-throated Blue Warbler. We converted the effect sizes on the logit scale with standardized distances to probabilities as a function of distance in km for SY versus ASY males.

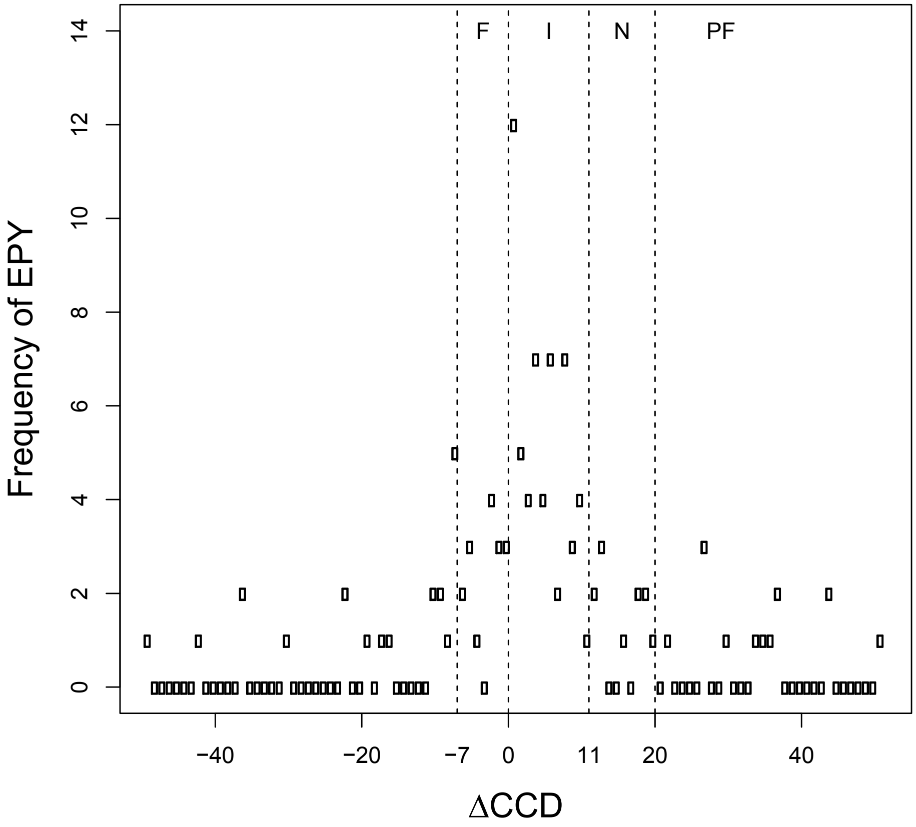

Figure 4.2. Frequency distribution of the difference in the clutch completion dates (ΔCCD) between a male's social female and his extra-pair female in the Black-throated Blue Warbler. Positive values indicates when the social female had completed her clutch before the extra-pair female or after (negative values). The vertical dashed lines indicate the onset and length of each nest stage (F = fertile, I = incubation, N = nestling, PF = post-fledging).

Table 4.1. Fixed effects from a GLMM [a] examining the spatial factors affecting the probability of a male siring extra-pair young (EPY) in a focal nest in Black-throated Blue Warblers at the Hubbard Brook Experimental Forest, NH. Significant terms are in bold.

	Probability of EPY		
Model term[b]	β ± SE	*F*	*P*
Distance [c]	-0.82 ± 0.31	-2.65	**<0.01**
Distance^2	0.72 ± 0.11	6.78	**<0.001**
Distance^3	-0.17 ± 0.07	-2.59	**<0.01**
Breeding density index	-0.04 ± 0.12	-0.29	0.77
Extra-pair sire age (SY)	-0.14 ± 0.42	-0.34	0.73
Social male age (SY)	0.23 ± 0.38	0.61	0.54
Distance x Breeding density index	-0.01 ± 0.09	-0.08	0.94
Distance x Extra-pair sire age (SY)	0.79 ± 0.24	3.23	**<0.01**
Distance x Social male age (SY)	-0.05 ± 0.23	-0.23	0.82
Extra-pair sire age (SY) x Social male age (SY)	-0.34 ± 0.46	-0.74	0.46

[a] The model included nest and candidate male identity as random effects

[b] Distance and breeding density index were standardized to have sample mean = 0 and sample variance = 1

[c] Distance = Euclidean distance between focal nests (female locations) and the centroids of candidate males' territories

Table 4.2. Fixed effects from a GLMM [a] examining the spatial and ecological factors associated with territory quality (caterpillar density and abundance) on the probability of a male siring extra-pair young (EPY) in a focal nest in Black-throated Blue Warblers at the Hubbard Brook Experimental Forest, NH. Significant terms are in bold.

	Probability of EPY		
Model term [b]	β ± SE	*F*	*P*
Distance [c]	-2.15 ± 0.27	-8.02	**<0.001**
Territory size	-0.17 ± 0.26	-0.68	0.50
Caterpillar density index [d]	-0.93 ± 0.30	-3.08	**<0.01**
Extra-pair sire age (SY)	0.13 ± 0.36	0.38	0.71
Distance x Territory size	-0.13 ± 0.21	-0.60	0.55
Distance x Caterpillar density index	-0.77 ± 0.23	-3.33	**<0.001**
Territory size x Caterpillar density index [d]	-0.35 ± 0.30	-1.16	0.24
Distance x Extra-pair sire age (SY)	1.27 ± 0.31	4.15	**<0.001**
Distance x Territory size x Caterpillar density index	-0.15 ± 0.24	-0.61	0.55

[a] The model included nest and candidate male identity as random effects

[b] Distance and breeding density index were standardized to have sample mean = 0 and sample variance = 1

[c] Distance = Euclidean distance between focal nests (female locations) and the centroids of candidate males' territories

[d] Territory size x Caterpillar density index = caterpillar abundance

Table 4.3. Fixed effects from a GLMM [a] examining the temporal factors affecting the probability of a male siring extra-pair young (EPY) in a focal nest in Black-throated Blue Warblers at the Hubbard Brook Experimental Forest, NH. Significant terms are in bold.

	Probability of EPY		
Model term [b]	β ± SE	*F*	*P*
Sign of ΔCCD [c]	1.22 ± 0.45	2.71	**<0.01**
ΔCCD	-0.04 ± 0.02	-2.17	**0.03**
Distance [d]	-1.99 ± 0.34	-5.79	**<0.001**
Extra-pair sire age (SY)	0.35 ± 0.34	1.02	0.31
Sign of ΔCCD x ΔCCD	0.01 ± 0.02	0.57	0.57
Sign of ΔCCD x Distance	0.18 ± 0.40	0.45	0.66
ΔCCD x Distance	-0.02 ± 0.01	-1.50	0.13
Distance x Extra-pair sire age (SY)	1.33 ± 0.30	4.44	**<0.001**

[a] The model included nest identity as a random effect

[b] ΔCCD and distance were were standardized to have sample mean = 0 and sample variance = 1

[c] Sign of ΔCCD indicates when the male's social female had completed her clutch before the extra-pair female (positive values = 1) or after (negative values = 0)

[d] Distance = Euclidean distance between focal nests (female locations) and the centroids of candidate males’ territories