

**SEX AND THE CITY: EFFECTS OF INDIVIDUAL CONDITION AND HABITAT
URBANIZATION ON MOUNTAIN CHICKADEE REPRODUCTIVE BEHAVIOUR**

by

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ABSTRACT

Variation in individual condition and habitat quality influence reproductive tactics in many songbird species. The goal of this thesis was to gain insight into the reproductive tactics utilized by mountain chickadees. Specifically, I asked how individual condition and habitat urbanization influence a female's likelihood to engage in extra-pair copulations or produce a sex-biased brood. Over three breeding seasons, I monitored mountain chickadees nests in urban and rural habitat, and determined nestling sex and paternity using genetic techniques. I found extra-pair paternity is common in the mountain chickadee and can be attributed to the good genes hypothesis. I also found tenuous evidence to suggest sex allocation occurs in this species in response to habitat urbanization. However, in general, habitat urbanization had little effect on mountain chickadee reproductive tactics, suggesting the relative quality of urban vs. rural habitat is a complex dynamic and further characterization of this system is necessary.

PREFACE

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The design, execution, and analysis of all experiments in this thesis were directed by E. Bonderud, thus, the introductory and concluding chapters are written in first person singular. However, to acknowledge the contributions of collaborators, the two data chapters that constitute the independent studies from the thesis are written in first person plural.

Chapter 2: Bonderud, E. S., Otter, K. A., Burg, T. M., Marini, K. L. D. & Reudink, M. W. (2017). Patterns of extra-pair paternity in the mountain chickadee.

Chapter 3: Bonderud, E. S., Otter, K. A., Murray, B. W., Burg, T. M., Marini, K. L. D. & Reudink, M. W. (2017). Effects of parental condition and nesting habitat on sex allocation in the mountain chickadee. – Behaviour. In press.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Urban ecology and songbirds

Our planet is rapidly becoming urbanized. Between 1970 and 2000, nearly 60,000 km² of land was converted to urban use, and this area is projected to increase by over 1.5 million km² by the year 2030 (Seto et al. 2011). Though developing countries are experiencing the greatest increase in urbanization, urban land area continues to rise in North America and Europe (Seto et al. 2011). In already-urbanized, developed countries, the populations are increasingly moving out of urban centres and re-settling in suburban areas. Suburban colonization is characterized by sprawling, lower-density settlement than urban, and consequently, cities in developed countries are growing faster in area than in population (Marzluff et al. 2001). As such, it is important we further our understanding of the ecological impacts of urbanization at both the community and species level in these regions.

Urbanization transforms landscapes and introduces novel features, resulting in both challenges (e.g., limited nest sites, novel predators) and benefits (e.g., increased food availability) not found in a species' native habitat (reviewed in Seress and Liker 2015). Due to the readiness of some species to occupy or adapt to urban landscapes, birds are perhaps the most studied system with regard to urban ecology. Urbanization is often associated with lower avian species diversity, but higher density of those species that thrive in urban habitat (Shochat 2004; Chace and Walsh 2006; Møller 2009; Møller et al. 2012). In addition, species richness tends to peak in suburban areas (i.e., intermediate levels of urbanization), a trend that may be attributed to moderate levels of anthropogenic disturbance creating various novel, heterogenic habitats (Blair 1996; Seress and Liker 2015). Urban habitats facilitate an average 30% increase in population density from rural habitats across avian species (Møller

et al. 2012); however, how a particular species fares in urban habitat is dependent upon multiple factors.

Species can be classified as ‘urban avoiders’, ‘urban exploiters’, or ‘urban adaptors’ based on their ability to inhabit urbanized landscapes (Blair 1996; Seress and Liker 2015). Urban avoiders are species that are sensitive to human disturbance and are found in highest densities (and sometimes exclusively) in their native habitats (Blair 1996; Seress and Liker 2015). Urban avoiders are often habitat specialists (e.g., insectivores), and are consequently unable to meet their specific needs with urban habitat (Seress and Liker 2015). Examples of urban avoiders include the western wood-peewee (*Contopus sordidulus*), and blue-grey gnatcatcher (*Poliophtila caerulea*), both of which are migratory insectivores (Blair 1996). Some species, however, thrive in urban habitat, like the rock dove (*Columba livia*) and house sparrow (*Passer domesticus*) (Blair 1996). These species, the urban exploiters, are able to adapt to and exploit the novel resources available in highly urbanized habitat. Urban exploiters are frequently introduced species and are also often dependent on anthropogenic food sources (e.g., bird feeders, disposed food) (Blair 1996; Seress and Liker 2015). The final category, urban adaptors, is comprised of species that inhabit interface or suburban habitat. These species utilize natural resources, but are also able to exploit the additional resources provided by moderate levels of disturbance (e.g., ornamental plants, bird feeders) (Blair 1996; Seress and Liker 2015). Urban adaptors are commonly omnivorous and cavity-nesting species (Seress and Liker 2015), and include common backyard birds like the American robin (*Turdus migratorius*), house finch (*Haemorrhous mexicanus*), white-breasted nuthatch (*Sitta carolinensis*), and chestnut-backed chickadee (*Poecile rufescens*) (Blair 1996).

Due to its potential effects on food availability, density and interspecific interactions, habitat urbanization may affect female perceptions, and consequently, reproductive

decisions. If urbanization results in increased population density, females may, for example, be more likely to encounter males and assess them as suitable extra-pair (EP) sires, and thus, may be more likely to engage in extra-pair copulations (EPCs) (Griffith et al. 2002).

Conversely, if urbanization decreases either density (Marzluff 1997) or a female's ability to assess male signals (LaZerte et al. 2015), EP matings may be reduced. Females commonly use male song to assess male condition/ quality and inform reproductive decisions. Like many other songbird species (Slabbekoorn 2013), male mountain chickadees (*Poecile gambeli*) increase their use of high-frequency songs during the dawn chorus to compensate for the high ambient noise levels characteristic of urban habitat (LaZerte et al. 2017). Still, urban noise has been shown to degrade acoustic signals and reduce their detectability (LaZerte et al. 2015), suggesting a female's ability to assess male condition may be reduced in urban habitat even with these adjustments to song. Relative food availability in urban habitat may also influence a female's reproductive decisions. If resources are plentiful and density (thus competition) is lower, urban habitat may influence brood sex ratios, as sons may have greater value under these favourable conditions (Trivers and Willard 1973).

1.2. Strategic investment in offspring

A variety of mating tactics are found across avian species, all with the common goal of maximizing individual reproductive success (Johnson and Burley 1998). Variation in avian mating tactics can be attributed to asymmetry in the cost of reproduction and variance in reproductive success between the sexes: females often invest more in offspring, but stand to gain less in total reproductive output than males (Trivers 1972). Both genders act in ways that maximize reproductive success; males should seek additional fertilizations, while females should invest time and energy to maximize offspring survival and reproduction

(Trivers 1972). To do so, females may, for example, engage in EPCs with males of high quality (Griffith et al. 2002; Westneat and Stewart 2003) or bias offspring sex ratios toward the gender with greater reproductive potential (Trivers and Willard 1973; Alonso-Alvarez 2006). Extra-pair paternity (EPP) and brood sex ratio bias are just two of the many ways females may increase reproductive success, but they will be the focus of this thesis.

1.2.1. Extra-pair paternity

The vast majority of songbird species are socially monogamous, with a single male and female pairing and caring for offspring (Westneat and Stewart 2003). Trivers (1972) suggested that males in socially monogamous species may participate in a mixed mating strategy of social monogamy while increasing reproductive success through EPCs with neighbouring females, siring offspring but withholding parental care. Likewise, socially monogamous females may increase reproductive success by engaging in EPCs (Trivers 1972). The advent of molecular tools for paternity analysis revealed these predicted strategies to be wide-spread (Birkhead and Møller 1992), with at least 70% of socially monogamous songbird species confirmed to be genetically promiscuous, and engage in EPP (Westneat and Stewart 2003).

There are several hypotheses as to why a female may choose to engage in EPCs including: to ensure fertilization of her eggs; to increase the genetic diversity or genetic quality of her offspring; or, to receive direct benefits, like defense or resources, from the EP male (Griffith et al. 2002). One hypothesis that has garnered significant attention is the good genes hypothesis (Birkhead and Møller 1992; Kempenaers and Dhondt 1993). The good genes hypothesis states that female engage in EPCs with males of greater genetic quality than their social male in order to obtain more favourable genes for their offspring (Kempenaers

and Dhondt 1993; Griffith et al. 2002). Under this hypothesis, females assess male condition through condition-dependent phenotypic cues (Kempnaers and Dhondt 1993; Griffith et al. 2002). In addition, habitat quality is also commonly hypothesized to influence rates of EPP. In low-quality habitats with limited food availability, EPP is predicted to be more prevalent, as females may engage in EPCs with males on higher-quality territories in order to gain increased access to food (Griffith et al. 2002). The presence of EPP in songbirds is commonly attributed to differences in mate condition/ quality (e.g., Kempnaers et al. 1992, 1997; Otter et al. 1998) or territory quality (e.g., Rubenstein 2007).

1.2.2. Sex allocation

Sex allocation theory predicts females should bias the sex ratio of their offspring in response to differences in the reproductive value of sons and daughters (Trivers and Willard 1973; Charnov 1982). Because males typically experience greater variance in reproductive success than females, a high-quality son may have the potential to produce more offspring and have higher reproductive success than a daughter of the same quality, while the opposite may be true for low-quality sons and daughters (Trivers and Willard 1973). Offspring reproductive value may vary as a result of both intrinsic factors (e.g., the female's own condition) and extrinsic factors (e.g., mate condition and habitat quality) (Trivers and Willard 1973; Burley 1981). Therefore, females should bias brood sex ratios in response to such attributes. In nature, biased sex allocation in response to parental condition and habitat quality has been documented in many vertebrates, including numerous bird species (reviewed in Alonso-Alvarez 2006).

The Trivers and Willard (1973) hypothesis of sex allocation predicts females in good condition should produce male-biased broods, assuming offspring condition and survival are

correlated with maternal condition. The Charnov model (1982) predicts females should alter sex allocation based on paternal attributes that may benefit one gender more than the other. The relationship between maternal condition and brood sex ratio has been well studied and supported in several avian species (Nager et al. 1999; Whittingham and Dunn 2000; Alonso-Alvarez and Velando 2003; Pike and Petrie 2005). Studies on songbird species have commonly considered the influence of male condition and habitat quality on offspring sex. For example, male mountain bluebirds (*Sialia currucoides*) display full-body UV-blue plumage, the colour of which is dependent on an individual's nutritional state at the time of moult (i.e., condition dependent) (Keyser and Hill 1999; Siefferman and Hill 2007; Doyle and Siefferman 2014). Female mountain bluebirds paired with more brightly-coloured males produce male-biased broods, suggesting females perceive and respond to variation in male colouration (i.e., condition) (Bonderud et al. 2016).

The physiological mechanisms allowing females to produce biased brood sex ratios at laying are poorly understood, however, the most plausible theories suggest female hormonal control (Pike and Petrie 2003; Navara 2013). Testosterone, for example, is present in greater amounts in the yolks of peafowl (*Pavo cristatus*) eggs containing male chicks, and has been suggested to promote the development of male eggs from the first meiotic division (Petrie et al. 2001). Female hormone levels change in response to mate attractiveness, self-condition and attractiveness, and resource availability, thus, these external factors may be reflected in ovarian and yolk hormone levels, resulting in sex specific follicle or embryo development (Pike and Petrie 2003).

1.3. Study species

Mountain chickadees are small, non-migratory songbirds common to the montane coniferous forests of western North America (McCallum et al. 1999). This highly adaptable species also commonly colonizes suburban habitat. Mountain chickadees are secondary cavity nesters, and will readily nest in artificial nest boxes. Chickadee social structure is governed by dominance hierarchies (McCallum et al. 1999; Ratcliffe et al. 2007). Although much of our understanding of this system comes from black-capped chickadees (*Poecile atricapillus*), mountain chickadees (*Poecile gambeli*) are known to form linear dominance hierarchies within winter flocks (McCallum et al. 1999), with males typically dominant to females, and adults typically dominant to juveniles (Grava et al. 2012). This is parallel to the social rank structure of black-capped chickadees; thus, it is likely that other predictors of dominance in mountain chickadees are similar to those known for black-capped chickadees, such as body condition (Schubert et al. 2007), and male song output (Otter et al. 1997).

In black-capped chickadees, dominant individuals gain increased access to resources (Ratcliffe et al. 2007) and are sought as both social mates (Otter and Ratcliffe 1996; Ramsay et al. 2000) and EP partners (Otter et al. 1994, 1998). Increased access to resources typically results in higher survivorship, and not surprisingly, age is a predictor of rank in black-capped chickadees (Otter et al. 1999; Schubert et al. 2007). Further, older birds are more likely to advance in rank (following the death of more dominant birds) or maintain high rank (Schubert et al. 2008) relative to younger birds. Males also signal their status through condition-dependent traits, which appear to be used by females to assess male quality (Otter et al. 1997; Mennill et al. 2003). In black-capped chickadees, male song output is a condition-dependent trait, with males in good condition (usually dominant males) singing for longer periods and at higher frequencies than males in poor condition (usually subordinate

males) (Otter et al. 1997; Grava et al. 2009). In a supplemental feeding experiment, Grava et al. (2009) found male black-capped chickadees that received additional food had greater song output than their unfed counterparts. This trend was observed in both dominant and subordinate males, and in both high- and low-quality habitats, suggesting individual condition is the main component contributing to variation in song output (Grava et al. 2009). Thus, the difference in song output between dominant and subordinate males appears to be a byproduct of differential resource accessibility.

Mountain chickadees can be classified as an ‘urban adaptor’ species, being common in both conifer forests and suburban habitat (Marini et al. 2017). Mountain chickadees present a unique opportunity to investigate the effects of urbanization on avian reproduction, as this species is native to coniferous forests (McCallum et al. 1999), rather than deciduous forests, as other focal species have been (Bailly et al. 2016). Compared to deciduous forest, urban habitat has a lower density of deciduous vegetation, and consequently, food availability for deciduous-specialists. Thus, urban habitat is often cited as lower-quality habitat for such species (Marzluff 1997; Blewett and Marzluff 2005). For species native to conifer forests, the opposite may be true. There is greater deciduous vegetation at my urban nest sites as compared to the conifer-dominated rural nest sites. Because deciduous trees typically bear greater insect abundance and diversity (Southwood 1961), it is possible urban habitat is associated with greater prey availability. Indeed, urban habitat appears to provide benefits (e.g., access to bird feeders, earlier leaf-out and insect emergence) to conifer-natives, as the mountain chickadees in my study population initiate breeding earlier in urban habitat (Marini et al. 2017). In addition, nestlings from urban nests have faster feather growth than their rural counterparts (Marini et al. 2017), which could indicate nestlings in urbanized areas

are being better provisioned, as has been shown in song sparrows (*Melospiza melodia*; Searcy et al. 2004).

1.4. Study site

I monitored mountain chickadees breeding in nest boxes in urban and rural areas of Kamloops, BC, Canada, during the 2014-2016 breeding seasons (May-July). Nest boxes were distributed throughout south Kamloops on a gradient of rural to urban habitat (Figure 1.1). Rural nest boxes were located in Kenna Cartwright Park, an approx. 8 km² wilderness area typical of the region's native habitat. Vegetation in the park consisted of mature ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga mensiesii*) interspersed with Great Basin grassland habitat (e.g., sagebrush, *Artemisia tridentate*; saskatoon, *Amelanchier alnifolia*; Poaceae spp.). Urban nest boxes were distributed over approx. 37 km² and located in various areas of south Kamloops, including the Thompson Rivers University campus and suburban habitat encompassing neighbourhood parks and backyards of participating citizens. The vegetation at these sites consisted primarily of immature Douglas fir trees and various species of native and non-native deciduous trees and shrubs (e.g., maple spp., *Acer* spp; mountain ash, *Sorbus* spp.; various fruit trees).

1.5. Habitat classification

Studies contrasting urban and rural habitat typically describe habitat using dichotomous categories ('urban' vs. 'rural') based on the investigator's judgment of the habitat. However, classifying habitats in suburban or interface habitat is often not so straightforward. To reduce subjectivity and increase resolution when dealing with these types of habitats, a more effective approach is to calculate a index based on ground cover (e.g., natural vegetation or

man-made structures) and use that index to describe the degree of habitat urbanization (LaZerte et al. 2017; Marini et al. 2017).

Following the protocols and scripts developed by LaZerte et al. (2017; available from: <https://github.com/steffilazerte/urbanization-index>), I used a combination of manual and automated methods to create a habitat index for the habitat surrounding each nest location. I used R (v3.3.2, R Core Team, 2016) to plot a 75 m radius around each nest box location (approximately the size of the average territory) in Google Earth (Google Inc., 2015). I then imported these aerial images into the image manipulation software GIMP (The GIMP Team, 2015), where I manually classified the buildings, pavement, native and non-native grasses (grassland vs. lawns), deciduous trees, and coniferous trees around each point location. I then grouped buildings and pavement into a single variable ('urban features'), and conducted a principal components analysis (PCA) in R to collapse the five variables into a single, continuous index of urbanization.

I retained the first principal component (PC1), which accounted for 68% of the total variation in habitat ground cover. Higher PC1 values corresponded to greater cover of coniferous trees and native grass cover, and less cover of deciduous trees, non-native grasses, and urban features (i.e., greater natural vegetation cover, greater 'rural-ness', lower 'urban-ness') (PC1 loadings: coniferous trees = 0.35, natural grasses = 0.40, deciduous trees = -0.46, non-native grasses = -0.50, urban features = -0.51).

Following methods similar to those used in the habitat index, I assessed only the vegetation at each nest location to determine vegetation type and cover, and proxy food availability. I obtained aerial images of each nest location from Google Earth, and using GIMP, manually classified the deciduous tree cover, coniferous tree cover, and other ground cover (e.g., grass, pavement) within a 75 m radius of the nest. I conducted a PCA in R to

collapse the three variables into a single value to describe vegetation cover. PC1 accounted for 81% of the total variation in vegetation cover. Higher PC1 values corresponded to greater coniferous tree cover and lower deciduous tree and other ground cover (i.e., greater canopy cover, greater coniferous content) (PC1 loadings: coniferous trees = 0.63, deciduous trees = -0.48, other ground cover = -0.61). Because deciduous trees typically bear greater insect abundance and diversity (Southwood 1961), higher PC1 values may correspond to lower prey availability or quality.

1.6. Thesis overview

The goal of this thesis is to increase our understanding of how social and environmental factors influence reproductive decisions in mountain chickadees. Specifically, I ask how mate condition and habitat urbanization influence rates of EPP and brood sex ratio bias. This thesis is organized into four chapters: the general introduction, given here, two data chapters, and a concluding chapter synthesizing the findings of the thesis. In Chapter 2, I describe the overall patterns of EPP in mountain chickadees, and investigate the influence of adult condition, habitat urbanization and food availability on EPP rates. In Chapter 3, I conduct a similar analysis, but asking how these factors influence brood sex ratio bias. Finally, in Chapter 4, I provide a general discussion in which we synthesize the finding of Chapters 2 and 3, relate these findings to previous literature, and discuss the implications of these findings and potential future directions.

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1.8. Figures

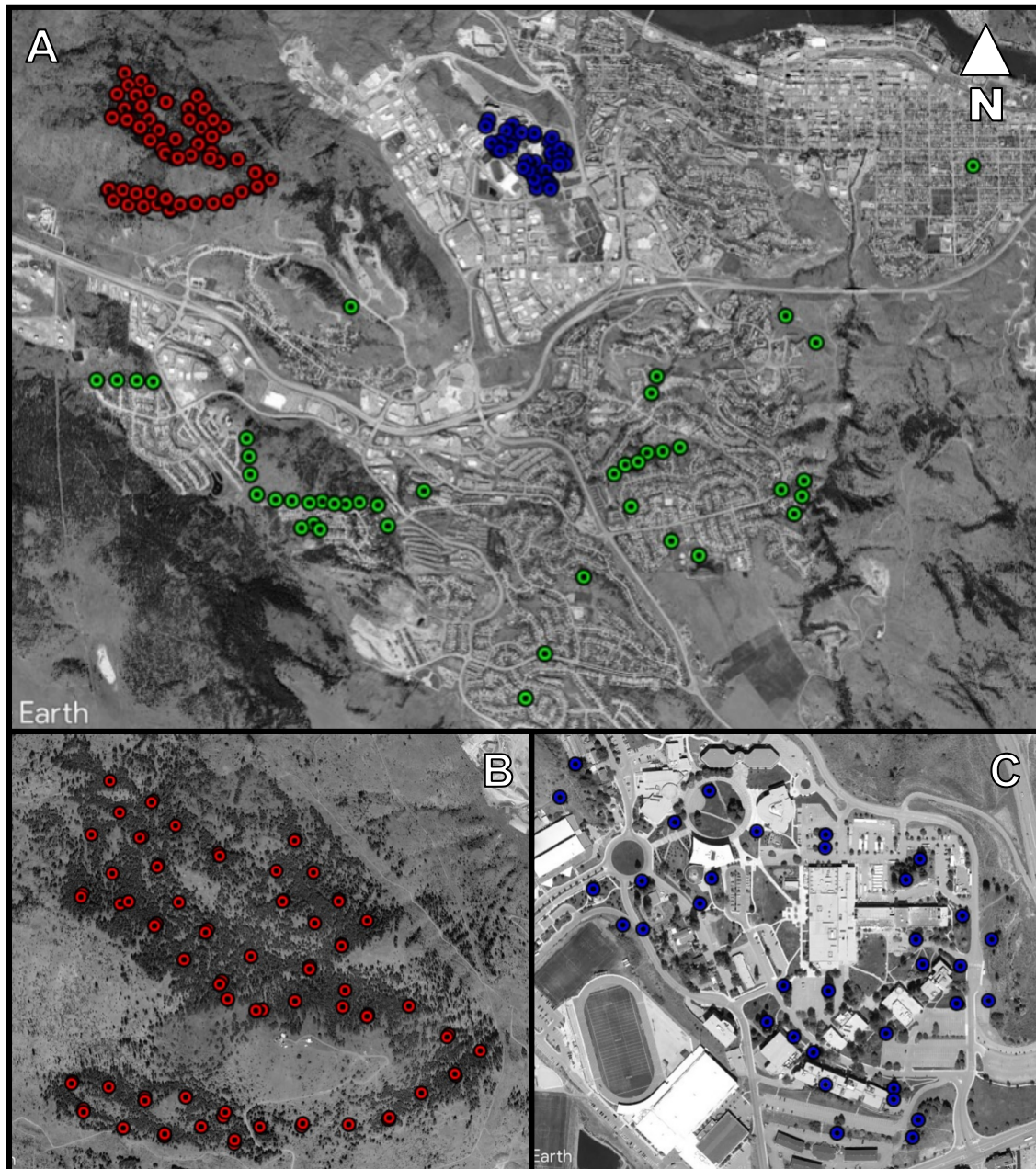


Figure 1.1. Locations of all mountain chickadee nest boxes in Kamloops, BC, Canada (box A). Nest boxes were distributed throughout south Kamloops on a habitat gradient from rural to urban. Locations included the conifer forests of Kenna Cartwright Park (i.e., rural; red points, box B), suburban neighbourhoods (green points; box A), and the Thompson Rivers University Campus (i.e., urban; blue points, box C).

CHAPTER 2: PATTERNS OF EXTRA-PAIR PATERNITY

2.1. Abstract

Extra-pair paternity is common in Paridae species and often attributed to the good genes hypothesis. In other songbird species, habitat quality influences EPP rates. Here, we ask how individual condition and habitat urbanization influence rates of EPP in mountain chickadees. We monitored mountain chickadee nests in urban and rural habitat, and determined parentage by genotyping individuals at 6 microsatellite loci. We found EPP is common in mountain chickadees, with extra-pair offspring (EPO) present in 44.4% of nests and accounting for 17.8% of offspring. We found support for the good genes hypothesis, with older males tending to raise fewer EPO, and females tending to engage in EPCs with older males. Parental provisioning effort was greater in broods containing a greater proportion of EPO. We did not find a significant effect of habitat on EPP rates, suggesting the impacts of urbanization on mountain chickadees may more complex than originally predicted.

2.2. Introduction

In birds, social monogamy is widespread, but is often coupled with a mixed-mating strategy that includes EPP. There are several hypotheses as to why a female may choose to engage in EPCs including: to insure fertilization of her eggs; increase the genetic diversity of her offspring; or, to receive direct benefits, like defense or resources, from the EP male (Griffith et al. 2002). Extra-pair paternity is common in the Paridae family (chickadees and titmice), with extra-pair offspring (EPO) present in 30-75% of nests, and accounting for 7-25% of offspring (Griffith et al. 2002). Among Parids, EPP is often explained by the good genes hypothesis: females engage in EPCs with males of higher quality than their social mates to

obtain favourable genes for their offspring. Females may assess male quality using phenotypic signals (e.g., plumage ornamentation, song, or behaviour) that convey information about physical condition (e.g., nutritional state) and/or genetic quality. For example, female blue tits (*Cyanistes caeruleus*) engage in EPCs with older, larger males (Kempnaers et al. 1997) and males with brighter ultraviolet blue plumage (Kempnaers et al. 1992), while female black-capped chickadees engage in EPCs with more dominant males (Smith 1988; Otter et al. 1994, 1998; Mennill et al. 2004).

Habitat urbanization can affect food availability and interspecific interactions, and consequently, may impact a female's likelihood to both seek and accept EPCs. Extra-pair paternity has been related to habitat quality in other songbird species. In house sparrows, experimentally increased food availability resulted in pairs spending more time together at the nest, which, in turn, lead to a five-fold reduction in EPP rates (Václav et al. 2003). In superb starlings (*Lamprotornis superbus*), Rubenstein (2007) found EPP to be less prevalent in higher-quality territories (i.e., greater vegetation cover and prey availability). As the author suggests, greater prey availability in high-quality territories may limit the distance females need to travel to forage, and consequently, decrease her probability of encountering an EP male. In contrast, serin (*Serinus serinus*) nests in territories with greater food availability are more likely to contain EPO than nests in poor-quality habitat (Hoi-Leitner et al. 1999). The authors postulate females on high-quality territories may be in better condition and more able to resist male mate guarding efforts, and thus, may have greater opportunity engage in EPCs (Hoi-Leitner et al. 1999).

For spotted towhees (*Pipilo maculatus*) breeding in urban parks, park edges are sites of high food abundance due to anthropogenic food sources (e.g., bird feeders), while interior habitat is comparatively lower-quality (Smith et al. 2016). If EPC rates decline with

increasing food abundance and decreased need for movement of individuals, EPP rates may be expected to be greater at the habitat interior than the edge. Smith et al. (2016), however, found the relationship between EPP rates and nest distance from habitat edge to vary non-linearly: the probability a nest contained EPO was the greatest at the habitat edge and interior, and lowest at intermediate distances. The authors suggest anthropogenic food sources may have drawn individuals from the interior habitat to the edge, resulting in increased contact with potential EP sires in edge territories and greater occurrences of EPP than predicted (Smith et al. 2016). In our study population, mountain chickadees in urban habitat are more dispersed than those in rural habitat (E. Bonderud, personal observation), suggesting any potential increase in food availability in urban habitat does not attract individuals from neighbouring rural habitat. As such, urban habitat may limit EPC opportunities for mountain chickadees.

Here, we investigate the ecological and social factors that influence patterns of EPP in mountain chickadees. Specifically, we ask whether female condition influences her propensity to engage in EPCs, and whether male condition predicts his likelihood of losing paternity. Following the good genes hypothesis, we predict females in good condition (presumably paired to a male in good condition) will be less likely to engage in EPCs, and males in good condition will be less likely to lose paternity. Finally, we ask how ecological characteristics of nesting habitat (urbanization, vegetation composition) affect rates of EPP. We predict EPP to be less prevalent in urban habitat (presumably higher-quality habitat) than rural habitat, though as shown by Smith et al. (2016), urban habitat may promote unexpected breeding strategies.

2.3. Methods

2.3.1. Field methods

We monitored nest boxes (Figure 1.1) every one to three days to identify the breeding pairs occupying boxes and measure breeding success. We caught adults at the nest and banded them with a Canadian Wildlife Service (CWS) aluminum leg band with a numerical identifier, and a unique combination of three coloured plastic leg bands. We classified age as either second-year (SY) or after-second-year (ASY) by examining the shape and wear of the outer retrices (Pyle 1997). We determined adult sex in the field by the presence (females) or absence (males) of a brood patch and by behaviour at the nest (e.g. only females incubate and only males sing), and later confirmed sex genetically (see Chapter 3 Section 3.3.3.2). For genetic analysis, we collected two tail feathers from each adult and stored the samples at -20°C . We measured adult fat score, weight, tail length, tarsus length, and flattened wing chord to evaluate individual body size and condition. To estimate overall body condition, we calculated the residuals from a mass x tarsus linear regression. Using the same metric in black-capped chickadees, Schubert et al. (2007) found leaner males with larger skeletal frames had higher dominance ranks. Thus, positive residual values suggest an individual is in better condition.

Six days after hatching, we banded nestlings with a single CWS aluminum band. Twelve days after hatching, we collected blood samples from nestlings by piercing the ulnar vein and drawing 10–20 μl blood into a micro-capillary tube. We stored blood samples dried on filter paper at -20°C .

2.3.2. *Dawn chorus recordings*

We recorded dawn choruses from males breeding in the urban ($n = 9$ unique males) and rural ($n = 10$ unique males) study sites between 4 May and 16 May during the 2014-2016 breeding seasons. We recorded choruses using a Sennheiser ME67/K6 microphone and either an Olympus LS-14 or a Marantz PMD670 digital recorder on settings of at least 44kHz sampling frequency and 16 bit digitization, or higher. We arrived on-site approximately 30 min before sunrise to determine chorus start time and obtain full chorus recordings. Male choruses were considered to be finished after a five-minute period of silence following the last vocalization. We analyzed recordings using Avisoft–SAS Lab Pro (Avisoft Bioacoustics) to measure variables known to be related to male condition in related species, including: total chorus length, maximum and average rates of vocalization, and song-to-call ratio.

2.3.3. *Parental care*

We conducted video recordings of parental provisioning trips to examine patterns of parental care at 14 nests during the 2016 breeding season. When nestlings were 12 days old, we recorded provisioning trips starting between 07:35 and 09:39 using a HD Hero2 or 3 (GoPro, CA, USA) for a 90 min period (mean \pm SD, 93 ± 17 min, $n = 14$). We attached the video recorder to either the tree the nest box was located on or a nearby tree, and aimed it at the nest box entrance. We manually analysed the recordings to determine the collective rate of provisioning by the attending male and female, as individual identity could not be determined in most videos. Provisioning rates were calculated as the number of trips to the nest per hour per nestling (Smiseth and Amundsen 2000). Adults were not captured at the nest within 48 h prior to recording to avoid modification of parental behaviour.

2.3.4. *Molecular methods*

We extracted total genomic DNA from feather samples using the standard protocol for the QIAamp DNA Micro Kit (Qiagen, Germany), and from blood samples using the standard protocol for the DNeasy Blood and Tissue Kit (Qiagen, Germany) with a modified lysis step.

We assessed nestling parentage using polymerase chain reaction (PCR) to genotype all adults and nestlings at six avian microsatellite loci (Table 2.1): Pat14 (Otter et al. 1998), Pat43 (Otter et al. 1998), Titgata02 (Wang et al. 2005), Titgata39 (Wang et al. 2005), Escu4 (Hanotte et al. 1994), and Escu6 (Hanotte et al. 1994). We amplified DNA in 10 μ l reactions containing 1X buffer, 1.5–2.5 mM MgCl₂ (1.5 mM: Pat43, Escu6; 2.0 mM: Pat14, Titgata39; 2.5 mM: Titgata02, Escu4), 0.25 U *Taq* DNA polymerase, 0.5 μ M forward primer, 1.0 μ M reverse primer, and 0.05 μ M fluorescently labeled M13 primer. All forward primers were synthesized with a M13 sequence on the 5' end to allow for incorporation of the fluorescently labeled M13 primer. We added 1% formamide to reactions involving Pat14 and Escu4.

We amplified all loci using a two-step annealing protocol: 1 cycle of 94 °C for 2 min, 50 °C for 45 s, and 72 °C for 1 min, followed by 7 cycles of 94 °C for 1 min, 50 °C for 30 s, and 72 °C for 45 s, followed by 25 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 45 s, followed by a final extension step of 72 °C for 5 min. For two loci (Escu4 and Escu6), the third step was increased from 25 to 31 cycles. For one locus (Escu4), we decreased annealing temperatures from 50 °C and 52 °C to 45 °C and 48 °C, respectively. PCR products were run on a 6% acrylamide gel on a Licor 4300 (Licor Inc.). We included individuals of known allele sizes, a negative control, and a 50-350 bp size standard on each load/channel to ensure alleles were sized consistently across gels.

2.3.5. *Parentage assignment*

We assigned parentage first by hand, and then again using CERVUS 3.0 (Kalinowski et al. 2007). In some cases we were not able to genotype individuals at all 6 loci due to insufficient quantities of DNA, which resulted in amplification failure. Previous paternity studies in black-capped chickadees employed only three microsatellite markers but still excluded sires with a high degree of confidence (Otter et al. 1998; Mennill et al. 2004). Thus, we only included nestlings with three or more loci successfully typed in parentage analysis (only one offspring was excluded for not meeting this criteria). We classified offspring as EP if they had two or more mismatches with the putative mother or father. We then conducted parentage analysis using CERVUS and combined these results with our manual assignments. CERVUS did not identify any additional EPO, however, some offspring classified as EP in our manual assignments were identified as within-pair by CERVUS. In these cases, if CERVUS identified the social male as the first or second most-likely father when all males in the population were considered, we accepted the CERVUS assignment; if not, we retained the manual assignment. In some instances CERVUS was unable to assign parentage due to an unknown putative male or female. In these cases, we used the manual assignment.

To identify EP sires, we used CERVUS to compare the genotypes of EPO to all males in the population. Using the males CERVUS identified as the most-likely fathers, we created an index of confidence in the assignment. Only males who held territory within 500 m (measured from box-to-box) of the female at some point during the study period were considered a likely match. Otter et al. (1998) found EP males typically hold the adjacent territory in black-capped chickadees, thus, we chose 500 m as a conservative cut-off distance because any father would have included males multiple territories away. We did not restrict our criteria to only males having bred in the same year as the female because not every male

was recaptured in subsequent years. If the male was not recaptured, we assumed he was still alive in subsequent breeding seasons, and that he bred in the vicinity of his original nest, as the mountain chickadees in our study sites have high site fidelity (E. Bonderud, personal observation).

2.3.6. *Statistical analyses*

Across all three breeding seasons, we monitored 46 nests and collected data on 260 nestlings and 59 adults. Of the 46 nests monitored, 31 had both the attending male and female identified, 5 had an unidentified female, 9 had an unidentified male, and 1 had neither adult identified. In total, 7 males and 7 females were recaptured in more than one breeding season. In three cases, the same male and female paired in more than one breeding season. In one case, a pair produced two successful broods within a single breeding season. We genotyped all 260 nestlings, and were able to assign maternity and paternity to all but two nestlings, one for which the paternal genotype was not known and the nestling was the only offspring in the brood (thus, we could not assign paternal alleles as coming from a WP or EP source), and the other for which only two loci amplified. In total, 258 nestlings (rural: $n = 161$, urban: $n = 97$) from 45 broods (rural: $n = 30$, urban: $n = 15$) were included in analysis.

We conducted statistical analyses in STATA 14 (StataCorp, 2017). To compare the condition of a male who lost paternity to the male who gained paternity in his nest (i.e., social male vs. EP sire), we conducted either a paired t-test (male body condition) or Wilcoxon signed-rank test (male age). In all other analyses, we analysed parentage data using generalized linear mixed models with logit link functions and binomial error distributions. To account for multiple observations of the same breeding adults, we included individual identity as a random effect in our models.

To ask whether rates of EPP differed between the study years, we used the number of EP offspring in the nest as the response variable, the total number of offspring in the nest as the binomial denominator, and study year as the predictor variable. Because 8 females produced more than one brood across the study period, and we assumed EPCs to be sought by females (rather than instigated by males), we included female identity as a random effect. We repeated the analysis twice, once using 2014 as the base comparison year and once using 2015, in order to compare all years to one another.

To ask how male condition predicts his likelihood to lose paternity in his own nest, we used nest paternity (no EPO present = 0, at least 1 EPO present = 1) as the response variable, age (ASY/SY) and body condition (mass x tarsus regression residual) as predictor variables and male identity as a random effect. We included ‘age x body condition’ as an interaction term, and dropped if non-significant ($p > 0.05$) to derive the final model. We ran a second model using male chorus rate and percent calls in chorus as predictor variables as chorus data was only available for a subset of males. To ask how male condition predicts the proportion of EPO in his nest, we constructed similar models, but used the number of EPO in the nest as the response variable and total number of offspring in the nest as the binomial denominator. To ask how female condition influences her propensity to engage in EPCs, we repeated the above analyses using measures of female condition (mass x tarsus regression residual, age) as the predictor variables, and female identity as a random effect.

To ask how habitat influenced the presence and proportion of EPO in nests, we constructed similar models using our habitat and vegetation indices as the predictor variables, and female identity as a random effect. Finally, to ask whether parental provisioning was adjusted in response to the presence or proportion of EPO in a nest, we constructed generalized linear models using parental provisioning rate (trips/hr/chick) as the response

variable, and nest paternity and the proportion of EPO in the nest as the predictor variables. We excluded the single case of renesting in the 2016 breeding season. We then isolated only those nests containing EPO, and re-ran the model including only the proportion of EPO in the nest as a predictor variable.

2.4. Results

2.4.1. General patterns of parentage

Overall, 17.8% (46/258) of offspring were sired by an EP male, and 44.4% (20/45) of nests contained EPO (Figure 2.1). The percentage of EPO within nests with mixed parentage ranged from 12.5% (1/8 offspring EP) to 100% (4/4 offspring EP). We were able to confidently identify 7 EP males from 6 (30.0%) of the mixed-paternity nests, with two EP males siring EPO within a single brood in one case. Both the social male and EP male were known within 4 nests, one of which was the nest with two EP males identified, resulting in a total of 5 social male/ EP male pairs for comparison (see below). We did not find the proportion of EPO to differ between study years, with the exception of the comparison between the 2015 and 2016 breeding seasons, in which there was a greater proportion of EPO in 2016 (2014 vs. 2015: $\chi^2 = -0.72, p = 0.47$; 2014 vs. 2016: $\chi^2 = 1.15, p = 0.25$; 2015 vs. 2016: $\chi^2 = 2.15, p = 0.03$).

All offspring were found to be the genetic offspring of their putative mother, with the exception of a single nestling fathered by the social male in a 2016 brood. Two females had been caught at the nest (one while incubating, one while provisioning) and both females had maternity in the brood. The female who had been caught while incubating (i.e., the social female) genetically matched 6/7 nestlings, while the female caught while provisioning was determined by CERVUS to be a better genetic match than the social female to the seventh

nestling, suggesting quasi-parasitism. Although CERVUS identified the second female as the most-likely mother, the nestling and the social female did not have any genetic mismatches (social female: 5/5 loci scored matched; 2nd female: 5/6 loci scored matched). In addition, the two females had very similar genotypes, matched the offspring at the same number of loci, and had similarly high likelihood scores. Together, these observations suggest the rejection of the social female as the genetic mother by CERVUS should be interpreted with caution, and this may not be a true case of quasi-parasitism. In two other cases, two females were caught attending a single nest, and in these instances, only one of the females held maternity in the brood.

2.4.2. *Condition and extra-pair paternity*

We did not find either female age or body condition to influence a female's likelihood to engage in EPCs or the proportion of EPO in her nest (all $p > 0.21$; Table 2.2). When we considered male condition, we did not find age, body condition, or chorus characteristics to influence a male's likelihood of losing paternity (all $p > 0.44$; Table 2.2). We also did not find male body condition or chorus characteristics to influence the proportion of EPO in the nest (all $p > 0.51$; Table 2.2). We did, however, find a near-significant ($p = 0.07$) effect of male age on the proportion of EPO in his nest: ASY males tended to have a lower proportion of EPO in their nests (Table 2.2).

2.4.3. *Social male vs. extra-pair male comparisons*

When we compared the condition of the male that lost paternity to the EP male, we found no difference in body condition ($t_4 = 1.38$, $p = 0.24$) or age ($W = -1.73$, $p = 0.08$) between the two, but the small sample size ($n = 5$) for these comparisons resulted in lower power. In all

five social male/ EP male pairs, the EP male was of equivalent age to the social male (ASY vs. ASY, $n = 2$), or older than (ASY vs. SY, $n = 3$). In the two comparisons where the males were of equivalent age, two males holding neighbouring territories on the Thompson Rivers University campus sired EPO in each other's nests (i.e., both gained paternity, but also both lost paternity to one another).

2.4.4. *Habitat effects*

We found neither the habitat index nor vegetation composition of nesting habitat to have an effect on either the presence or proportion of EPO in a nest (all $p > 0.09$; Table 2.2, Figure 2.2).

2.4.5. *Extra-pair paternity and parental care*

While nests containing EPO tended, overall, to be provisioned at a lower rate than those without, (though not significantly: $p = 0.06$; Table 2.3), provisioning rates were positively related to the proportion of offspring in a nest that were EP ($p = 0.007$; Table 2.3, Figure 2.3). When we isolated nests containing EPO, the relationship between provisioning rate and the proportion of EPO in the nest remained significant ($p = 0.006$; Table 2.3, Figure 2.3).

2.5. Discussion

Extra-pair paternity has been well-studied in Paridae species, but has, until now, not been investigated in mountain chickadees. Here, we present evidence that mountain chickadees also frequently engage in EPCs: EPO were found in almost 50% of nests and represented almost 20% of offspring. These were somewhat surprisingly high frequencies, as rates of EPP in the closely related black-capped chickadee tend around 30% of nests and 10-15% of

offspring (Otter et al. 1998; Ramsay et al. 2000; Mennill et al. 2004). However, similarly high, and even higher, rates of EPP to what we observed have been documented in the related blue tit (60% of nests; Delhey et al. 2007) and great tit (*Parus major*, 40% of nests; Lubjuhn et al. 1999). In addition, high rates of EPP have been observed in black-capped chickadees breeding in hybrid zones with Carolina chickadees (*Poecile carolinensis*) (55.6% of nests; Reudink et al. 2006) and mountain chickadees (62.5% of nests Grava et al. 2012).

The presence of EPP in Paridae species has often been attributed to the good genes hypothesis: females engage in EPCs to obtain more favourable genes for their offspring than their social mate can provide to increase their own fitness. In black-capped chickadees, females engage in EPCs with males of higher dominance rank than their social male (Otter et al. 1998; Mennill et al. 2004). In blue tits, several measures of condition appear to influence the decisions of females, with larger males (Kempnaers et al. 1997), older males (Kempnaers et al. 1997), males with greater song output (Kempnaers et al. 1997), and male who begin singing earlier (Poesel et al. 2006) being sought as EP sires. Here, we asked how male condition, as measured by age, weight relative to body size, and dawn chorus output influence his likelihood to lose paternity. We did not find any measures of male condition to predict the presence of EPO in his nest, nor did we find any significant relationship between male condition and the proportion of EPO in his nest. We did, however, find a near significant ($p = 0.07$) effect of male age and the proportion of EPO in his nest. Older, ASY males tended to raise fewer EPO, a finding consistent with the good genes hypothesis. In addition, we found that in all cases where both the social male and EP male were known, the EP male was either of equivalent age to the social male or older. In the cases where the males were of equivalent age, both males were ASY, though their age in years was not known. Together, these anecdotes suggest female mountain chickadees engage in EPCs with adult

males (5/5 cases), and males older than their social male (3/5 confirmed cases) – findings again consistent with the good genes hypothesis.

We found mountain chickadees adjust provisioning effort in relation to nest paternity. We found a strong positive relationship between provisioning effort and the proportion of EPO in a nest. Because it was difficult to discern individual identity in our nest watch videos, we assessed provisioning rate as the collective provisioning effort of both the male and female. If females are adjusting their provisioning, this could present a case of differential allocation (Burley 1986), with females increasing provisioning because they ‘know’ there are some potentially higher-quality EPO in their nest. Alternatively, if males are the ones increasing their parental effort, this could be a situation like in house finches where, in some populations, yellow males (less preferred by females) provision offspring at a higher rate (Badyaev and Hill 2002). However, not knowing who is doing the provisioning makes it difficult to interpret the pattern.

In contrast to multiple other studies, we failed to find a significant effect of habitat on EPP in mountain chickadees. Several studies investigating habitat and EPP have considered differences in food availability in otherwise similar habitat as the determinant of habitat quality (Hoi-Leitner et al. 1999; Václav et al. 2003; Rubenstein 2007). Here, we asked how EPP varied along a gradient from rural to urban habitat. Unlike other studies, we had no direct measure of habitat quality (e.g., food availability). However, urban habitat appears to be better quality than rural habitat to the mountain chickadees in our study population (Marini et al. 2017), presumably because of greater food availability due to the presence of bird feeders and greater deciduous tree content. Still, we did not find EPP to be related to the habitat index or vegetation composition of nesting sites, indices that were derived to describe

habitat urbanization and quality, and vegetation composition and food availability, respectively.

Other studies with sites bordering urban and suburban neighbourhoods have found effects of urbanization on EPP. For spotted towhees breeding in urban parks, EPP rates are highest in interior habitat and urban-bordering edge habitat, and lowest at intermediate distances from the edge (Smith et al. 2016). In these parks, edge habitat is comparatively better quality than interior habitat, as there is greater food abundance at edges due to anthropogenic food sources (Smith et al. 2016). As such, the high prevalence of EPP in interior territories was expected, but the same observation in edge territories was not. The authors suggest additional food sources in edge habitat drew individuals from the surrounding lower-quality habitat, resulting in higher densities of transient birds in the edge habitat and increased instances of EPCs (Smith et al. 2016). In blue tits, Kempenaers et al. (2010) found artificial night lighting (i.e., street lights) in suburban habitat influences EPP. Compared to males with territories in interior forest, males in edge habitat bordering lighted suburban neighbourhoods were not in any better condition, but were more successful at gaining paternity in other nests (Kempenaers et al. 2010). Males in lighted territories also began singing earlier (Kempenaers et al. 2010). In natural, forest habitat, female blue tits engage in EPCs with early-singing males, suggesting the timing of dawn singing may be an indicator of male quality (Kempenaers et al. 1992). Thus, females may have perceived early-singing males in suburban habitat as being high quality and, consequently, sought these males as EP partners.

Like in our study population (Marini et al. 2017), Kempenaers et al. (2010) found females nesting in suburban territories began laying eggs earlier. Females should time breeding so that peak nestling food demand aligns with peak food availability, however, as

the authors suggest, earlier laying may have led to a mismatch between the two in suburban habitat (Kempnaers et al. 2010). Thus, rather than indicating better habitat quality, as we had speculated, earlier clutch initiation in urban habitat may be a maladaptive behaviour instigated by features of urban habitat (e.g., artificial lighting). Investigations of the relative timing of breeding in relation to insect abundance peaks in either habitat would have to be conducted to discern whether these mismatches occur. Together, these examples illustrate the complex dynamics of urban habitat, and suggest the differences we have previously observed between mountain chickadees in urban and rural habitat (see Marini et al. 2017) may represent more than simply differences in habitat quality.

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2.7. Tables

Table 2.1. Allelic variation at the 6 microsatellite markers. Size ranges for microsatellite alleles are given in base pairs, along with the total number of unique alleles observed, and observed (H_o) and expected (H_e) heterozygosities.

Locus	Size (bp)	# Alleles	H_o	H_e
Pat14	132-176	20	0.93	0.86
Pat43	158-232	16	0.88	0.85
Titgata02	222-270	12	0.82	0.80
Titgata39	210-238	11	0.89	0.87
Escu4	170-184	8	0.70	0.76
Escu6	128-152	13	0.91	0.85

Table 2.2. Results of generalized linear mixed models asking how male condition (age, mass x tarsus regression residual, chorus characteristics), female condition (age, mass x tarsus regression residual), and habitat characteristics (habitat index, vegetation index) influence the presence or proportion of EPO in nests.

Variable	Estimate	SE	χ^2	<i>p</i>	<i>n</i> (broods)
Female condition models					
Presence of EPY in nest					
Female age	-0.06	1.40	-0.04	0.97	38
Female body condition score	0.45	0.82	0.55	0.58	38
Proportion of offspring EP					
Female age	0.27	0.94	0.29	0.77	38
Female body condition score	0.59	0.47	1.25	0.21	38
Male condition models					
Presence of EPY in nest					
Male age	0.05	0.85	0.05	0.96	36
Male body condition score	-0.05	0.63	-0.08	0.94	36
Proportion of offspring EP					
Male age	-1.65	0.91	-1.82	0.07	36
Male body condition score	-0.44	0.86	-0.50	0.62	36
Male chorus rate					
% calls in chorus	-0.12	0.15	-0.78	0.44	24
Proportion of offspring EP					
% calls in chorus	0.01	0.02	0.75	0.45	24
Habitat models					
Presence of EPY in nest					
Habitat index	-1.13	1.27	-0.89	0.37	45
Vegetation index	1.16	1.35	0.86	0.39	45
Proportion of offspring EP					
Habitat index	-0.88	0.52	-1.68	0.09	45
Vegetation index	0.90	0.57	1.58	0.12	45

Table 2.3. Results of generalized linear models asking whether parental provisioning is adjusted in response to the presence or proportion of EPO in nests.

Variable	Estimate	SE	χ^2	p	n (broods)
All nests					
Nest paternity	-1.42	0.75	-1.91	0.06	12
Proportion EPO	3.11	1.16	2.68	0.007*	12
Mixed paternity nests only					
Proportion EPO	3.11	1.14	2.74	0.006*	7

* Significant value, $p < 0.05$

2.8. Figures

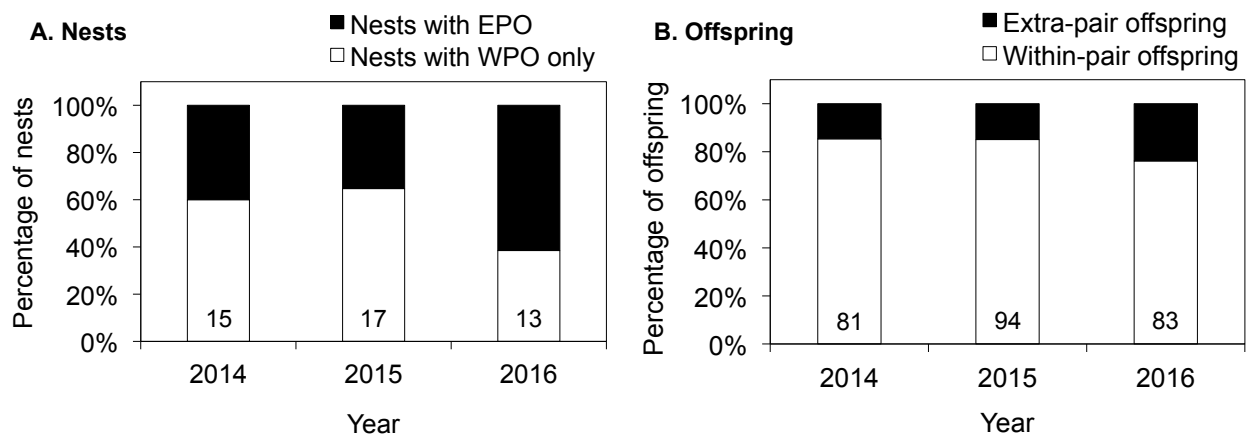


Figure 2.1. (A) Percentage of nests containing at least one extra-pair offspring (EPO) and all within-pair offspring (WPO); (B) Percentage of offspring that were extra-pair and within-pair. Numbers within bars represents total number of (A) nests and (B) offspring sampled in each study year.

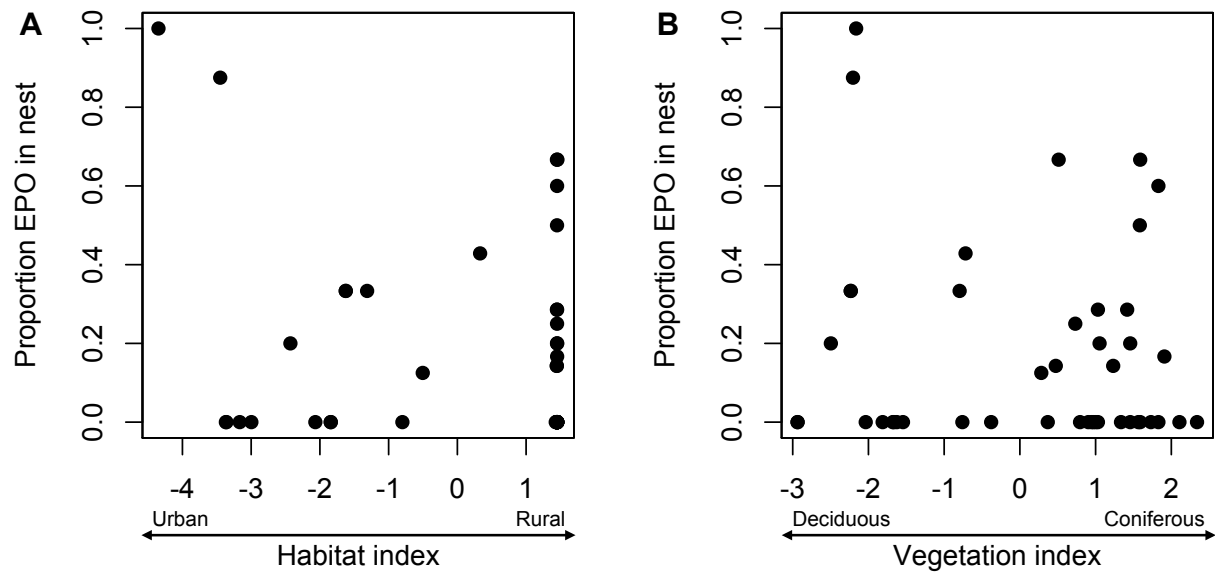


Figure 2.2. The proportion of EPO in a nest was not influenced by either the (A) habitat index or (B) vegetation index of the nesting habitat. These indices were derived to describe (A) the degree of habitat urbanization and overall quality, and (B) vegetation composition, canopy cover and food availability.

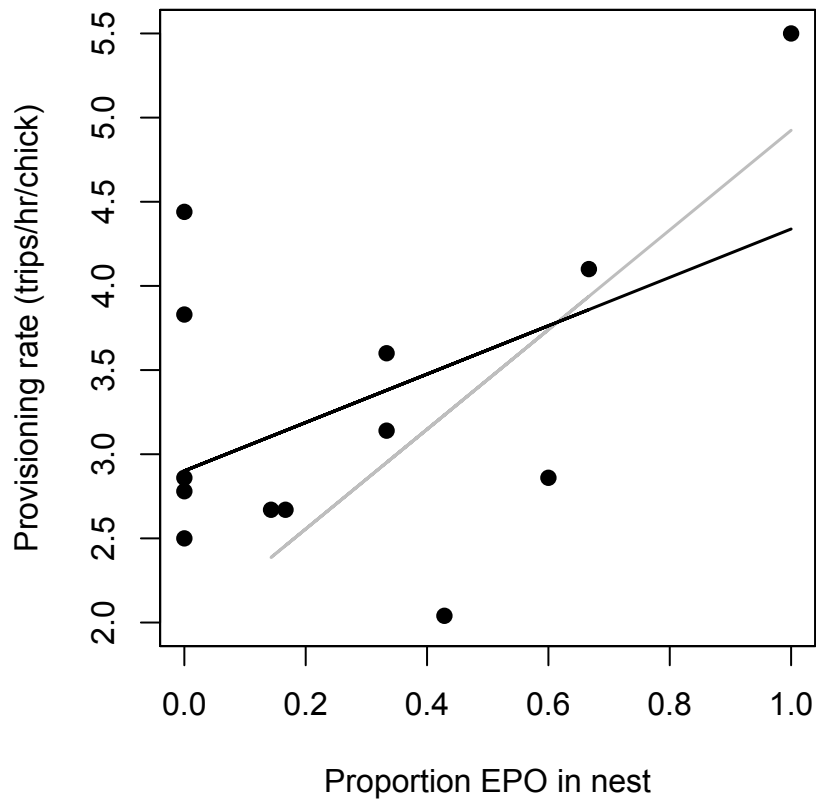


Figure 2.3. Nests containing a greater proportion of EPO were provisioned at higher rates. This relationship was significant when both nests with and without EPO were considered ($p = 0.007$; black line), and remained significant when we isolated nests containing EPO ($p = 0.006$; grey line).

CHAPTER 3: EFFECTS OF PARENTAL CONDITION AND URBANIZATION ON SEX ALLOCATION

3.1. Abstract

When the reproductive value of sons vs. daughters differs, sex allocation theory predicts females should bias the sex ratio of their broods towards the higher-value sex. Females in numerous bird species appear to bias offspring sex in response to self and mate condition, and breeding habitat quality. Over three breeding seasons, we monitored mountain chickadees breeding along a rural to urban habitat gradient. We did not find female condition or the condition of the putative father or true genetic father to influence offspring sex. We found marginal evidence for sex allocation in relation to habitat urbanization, though opposite to our predictions. In urban habitat, offspring were more likely to be female as the degree of habitat urbanization increased. We suggest habitat quality may be influential in mountain chickadee reproductive decisions; however, the ecology of mountain chickadees may not fulfill the assumptions of sex allocation theory.

3.2. Introduction

Sex allocation theory predicts females should bias the sex ratio of their offspring in response to differences in the reproductive value of sons and daughters (Trivers and Willard 1973; Charnov 1982). Offspring reproductive value may vary as a result of both intrinsic factors (e.g., the female's own condition) and extrinsic factors (e.g., mate condition and habitat quality) (Trivers and Willard 1973; Burley 1981). Therefore, females should bias brood sex ratios in response to such attributes. In nature, biased sex allocation in response to parental

condition and habitat quality has been documented in many vertebrates, including numerous bird species (reviewed in Alonso-Alvarez 2006).

Studies on songbird species have commonly considered the influence of male condition and habitat quality on offspring sex. Chickadee social structure is governed by dominance hierarchies (McCallum et al. 1999; Ratcliffe et al. 2007). In black-capped chickadees, dominant individuals gain increased access to resources (Ratcliffe et al. 2007) and are sought as both social mates (Otter and Ratcliffe 1996; Ramsay et al. 2000) and EP partners (Otter et al. 1994, 1998). Males signal their status through condition-dependent traits, which appear to be used by females to assess male quality (Otter et al. 1997; Mennill et al. 2003). Thus, females may be able to strategically gauge the value of producing male vs. female offspring on the relative rank/age or perceived condition of their mate.

Habitat urbanization may affect female perceptions, though, due to its potential effect on food availability, density and interspecific interactions. The influence of habitat quality on offspring sex ratios has been demonstrated in the great tit, with sex ratios shifting toward male-biased in high-quality habitats (Stauss et al. 2005). In the blue tit, Bell et al. (2014) found variation in offspring sex ratio to be associated with an interaction between male quality and territory quality, with only broods in high-quality breeding territories showing a relationship between sex ratio and male quality. Thus, habitat quality may have both direct and indirect effects on offspring sex.

We studied mountain chickadees breeding in rural and urban habitat over three breeding seasons to ask whether female mountain chickadees produce sex-biased broods in response to mate condition or nesting habitat. Although previous work in the congeneric black-capped chickadee found no evidence for sex allocation with regard to male condition, female condition, or paternity (Ramsay et al. 2003), offspring sex ratios have been positively

correlated with habitat quality (as described above) and male condition/quality (e.g., Kölliker et al. 1999; Sheldon et al. 1999; Stauss et al. 2005) in other Paridae species. Thus, we suggest there is potential for these factors to influence brood sex ratios in mountain chickadees. In accordance with Charnov's (1982) model of sex allocation, we predicted females paired with males in better condition (older, larger) would produce male-biased broods. Under the Trivers and Willard (1973) hypothesis, we predicted females in better condition (older, larger) would produce male-biased broods. In addition, we asked whether habitat urbanization affects brood sex ratios, and predicted nests in urban habitat (presumably better quality habitat) to be male-biased.

3.3. Methods

3.3.1. Field methods

See Chapter 2 Section 2.2.1.

3.3.2. Parental care

See Chapter 2 Section 2.2.3.

3.3.3. Molecular methods

3.3.3.1. DNA extraction

See Chapter 2 Section 2.2.4.

3.3.3.2. Molecular sexing

We determined adult and nestling sex via PCR using the P8/P2 primer set to amplify the chromo helicase DNA-binding genes of the Z and W sex chromosomes (Griffiths et al.

1998). In mountain chickadees, this reaction yields a 350 bp product from the Z chromosome (present in both males and females) and a 450 bp product from the W chromosome (present only in females). PCR amplification was carried out in a total volume of 25 µl using the standard reaction and cycling conditions for the Multiplex PCR Kit (Qiagen, Germany). The cycling conditions were as follows: an initial heat activation at 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 48°C for 90 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min. We separated PCR products in 2.5% agarose gels run at 90 V for 60 to 90 min. Individuals of known sex, a negative control and a 50-500 bp size standard were run on all gels. All gels were manually scored by a single observer.

3.3.3.3. Parentage assignment

See Chapter 2 Section 2.2.5.

3.3.4. *Statistical analyses*

Across all three breeding seasons, 46 nests were monitored, providing data on 266 nestlings and 59 unique adults. Hatching success in our population was 87% (266/306 eggs hatched). Of the 266 nestlings, we were able to obtain genetic samples from 260 (98%) nestlings from 46 broods. Genetic samples were not obtained from 6 nestlings from 6 broods because mortality occurred before collection on day 12. Thus, our data closely represent primary sex ratios, but more accurately represent sex ratios at hatching.

Of the 46 nests monitored, 31 had both the attending male and female identified, 5 had an unidentified female, 9 had an unidentified male, and 1 had neither adult identified. In total, 7 males and 7 females were recaptured in more than one breeding season. In three cases

where both the male and female in the social pair were known, the same individuals paired in more than one breeding season. In one case, a pair produced two successful broods within a single breeding season.

We refer to population sex ratio as the number of male nestlings in the population over the total number of nestlings in the population; brood sex ratios are the number of male nestlings in a brood over the total number of nestlings in that brood (i.e., a ratio of 1 indicates all males, while a ratio of 0 indicates all females).

Statistical analyses were conducted using STATA 14 (StataCorp, 2017). Because our nestling sex ratio data were in proportional form and did not meet the assumptions of normality, we used the non-parametric Wilcoxon signed-rank test to determine whether sex ratios deviated from unity. To avoid pseudo-replication due to seven females having broods in multiple breeding years, we performed this analysis by year and excluded the single case of renesting in 2016. In all other analyses, nestling sex data were analyzed using generalized linear mixed models with binomial error distributions and logit link functions. We included brood identity nested within female identity as a random effect in all models. Broods were nested within female identity to account for second broods and variation at the level of the mother, as seven females produced more than one brood across the three study years.

To ask whether brood sex ratios differed between study years, we used the number of male offspring in the nest as the response variable, the total number of offspring in the nest as the binomial denominator, and study year as the predictor variable. We repeated the analysis twice, once using 2014 as the base comparison year and once using 2015, in order to compare all years to one another. We did not find that brood sex ratios differed between years (2014 vs. 2015: $\chi^2 = 0.67$, $p = 0.50$; 2014 vs. 2016: $\chi^2 = -0.92$, $p = 0.36$; 2015 vs. 2016: $\chi^2 = -1.61$, $p = 0.11$), so data were pooled for subsequent analyses.

To ask how individual condition and habitat characteristics predict nestling sex, we constructed models using nestling sex (male = 1, female = 0) as the response variable. We also included nestling paternity as a categorical predictor variable to account for nestlings having different parentage (within-pair vs. extra-pair paternity). We chose this model set-up to understand how the variables of interest predict individual nestling sex within a brood, rather than the overall brood sex ratio (e.g., nestlings more likely to be male when male in better condition, thus brood sex ratio male biased). We included ‘condition variable x age’ and as interaction terms in condition models, and removed non-significant ($p > 0.05$) interactions to derive the final models.

To ask whether male condition predicted nestling sex, we constructed a model using male age (SY = 0, ASY = 1) and male body condition (mass x tarsus regression residual) as predictor variables. We repeated the analysis twice, first testing characters of the putative father (i.e., social male) and again with the true genetic father. For models including the genetic father, only those males for which we had the highest degree of confidence in the assignment were included. When males for which we had lower confidence in the assignment were included, model significance was reduced, suggesting the additional assignments may be erroneous. In addition, we asked whether female condition predicted nestling sex using similar models with female age and female body condition (mass x tarsus regression residual) as predictor variables. To ask whether habitat characteristics predicted nestling sex, we constructed a model using urbanization and tree type indices as predictor variables.

To ask whether nestling sex varied with parentage, we constructed a model using nestling paternity (WP = 0, EP = 1) as the predictor variable. We repeated this analysis twice, once including all nestlings, and again using only nestlings from broods containing extra-pair young.

Finally, to ask whether parents adjusted provisioning rates in response to offspring sex ratio or habitat characteristics, we constructed a generalized linear model with parental provisioning rate (trips/hr/chick) as the response variable, and brood sex ratio, urbanization index and tree-type index as the predictor variables. We excluded the single case of renesting in the 2016 breeding season.

3.4. Results

3.4.1. *Population and brood sex ratios*

Broods ranged in size from 1 to 8 nestlings (mean \pm SD, 5.9 ± 1.5). Brood sex ratios ranged from 0.17 (almost exclusively female) to 1.0 (exclusively male) (Figure 3.1). Neither population nor mean brood sex ratios were found to deviate significantly from 0.5 in any of the three study years (Table 3.1).

3.4.2. *Adult condition and nesting sex*

We found neither body condition (residual of mass to tarsus) nor age of either the social male or genetic father predicted nestling sex (all $p > 0.25$; Table 3.2). In addition, we found neither female body condition nor age predicted nestling sex (all $p > 0.15$; Table 3.3).

3.4.3. *Parentage and nestling sex*

Paternity was assigned to all offspring with DNA collected, with the exception of two nestlings ($n = 258$), one for which a putative paternal genotype was not known and the nestling was the only offspring in the brood (thus, we could not assign paternal alleles as coming from a WP or EP source), and the other that had only 2 loci amplify. Extra-pair offspring were observed in 44.4% of nests and accounted for 17.8% of offspring. We did not

find paternity to have an effect on offspring sex, neither when we considered all nestlings ($\chi^2 = -1.03, p = 0.30$) nor when we isolated those from nests with mixed paternity ($\chi^2 = -1.33, p = 0.19$).

3.4.4. *Habitat and nestling sex*

Neither the habitat index ($\chi^2 = 1.12, p = 0.26$) nor vegetation composition ($\chi^2 = -1.47, p = 0.15$) of nesting habitat had an effect on nestling sex. Upon visual inspection of the data, we noticed nests from rural (all nests in Kenna Cartwright Park) and urban (all other nests) habitat formed two distinct groups (Figure 3.2). We separated the data by habitat category and found habitat index influenced nestling sex in the urban ($\chi^2 = 2.00, p = 0.05$), but not rural ($\chi^2 = -1.00, p = 0.32$), habitat. In urban habitat, nestlings were more likely to be male with increasing habitat index values (i.e., increasing conifer cover, decreasing urban feature cover).

3.4.5. *Brood sex ratio and parental care*

Finally, we asked if parents adjust their provisioning in response to brood sex ratio or habitat characteristics. We found no effect of brood sex ratio on parental provisioning rates ($\chi^2 = 0.90, p = 0.37$). We also found no effect of habitat index ($\chi^2 = -1.45, p = 0.15$) or vegetation composition ($\chi^2 = 1.26, p = 0.21$) on provisioning rates.

3.5. Discussion

Over three breeding seasons, we found limited evidence for sex allocation in mountain chickadees. Habitat influenced offspring sex in urban habitat, though opposite to our predictions: offspring in urban habitat were more likely to be female as the habitat became

more urbanized. We did not find female condition or the condition of either the putative father (social male) or true genetic father to influence offspring sex. In addition, we did not find nestling sex to be related to paternity, or for parents to adjust provisioning effort in response to brood sex ratio. Together, these findings suggest the assumptions of sex allocation theory may not hold true for mountain chickadees.

Because males in good condition are expected to have higher reproductive value than females under both models of sex allocation (Trivers and Willard 1973; Charnov 1982), we predicted urban habitat and habitat with greater deciduous content may promote the production of male offspring. Previous work in our study population found mountain chickadees breeding in urban habitat initiate egg-laying earlier and rear faster-growing nestlings than their rural counterparts (Marini et al. 2017), suggesting urban habitat may provide benefits to this species. As proposed by Marini et al. (2017), urban nest sites may provide greater food availability to mountain chickadees through the presence of bird feeders. In addition, urban habitat is associated with greater deciduous tree abundance. Although mountain chickadees are native to coniferous forests, deciduous trees may host a greater abundance of the Arachnid and Lepidoptera species mountain chickadees forage (Southwood 1961).

Overall, we found neither the habitat index nor vegetation composition of nest sites influenced offspring sex. However, when we isolated nests occurring across a gradient of urban habitat, we found the habitat indices of these nests influenced nestling sex. In urban sites, offspring were more likely to be female with decreasing habitat index values, which are associated with decreasing conifer and native grass cover, and increasing cover of urban features and deciduous trees (i.e., increasing ‘urban-ness’). While brood sex ratios in the rural habitat varied across the same range as those in the urban, there was little to no variation in

the habitat indices of our rural sites. Thus, there is little variation in habitat gradients among nests in our rural sites for which female mountain chickadees could adaptively adjust offspring sex. By comparison, the habitat at our urban sites varies much more between nests, and so, this gradient may allow for strategic female response in sex-biasing of broods. The relationship between habitat urbanization and offspring sex was opposite to our predictions based on the findings of Marini et al. (2017). Thus, how mountain chickadees perceive the relative quality of urban vs. rural habitat, and the males breeding within them, may be complex.

Previous work in black-capped chickadees found no evidence for sex allocation, and suggested the assumptions of sex allocation theory may not hold in this species (Ramsay et al. 2003). It is possible the same is true for our study population of mountain chickadees. The Trivers and Willard (1973) hypothesis assumes that offspring condition is correlated to maternal condition during breeding. However, Trivers and Willard (1973) suggest this assumption may only be true for species with small, predictable brood sizes. The mountain chickadees in our study population have large, highly variable brood sizes (range 1 to 8 nestlings), thus, this assumption may not hold. The Charnov model (1982) assumes the reproductive value of sons vs. daughters is related to paternal attributes that differentially benefit the sexes. Based on this model, we predicted male condition might have an effect on nestling sex. We also predicted that extra-pair offspring might be more likely to be male, as female chickadees are known to seek better-quality males as extra-pair partners (Otter et al. 1998). However, we found no effect regarding either of these predictions, suggesting the estimates of condition we tested may either not influence female reproductive decisions, or may not differentially benefit one sex over the other.

Both models of sex allocation (Trivers and Willard 1973; Charnov 1982) assume the production of female offspring is disadvantageous under favourable conditions. Like males, female chickadees also establish dominance hierarchies (Ramsay and Ratcliffe 2003; Grava et al. 2012) and, therefore, there could be an advantage to older, potentially higher-ranking, females producing more female offspring. High-ranking females typically pair with high-ranking males (Smith 1976; Otter et al. 1999), and in turn, may gain increased access to resources and higher survivorship. However, the physiological and morphological determinants of female dominance hierarchies remain largely unknown (Ramsay and Ratcliffe 2003), making any effect of female dominance on sex allocation speculative.

While tenuous, our findings suggest habitat may be an important influence on offspring sex in mountain chickadees. The conclusions we can draw from these findings are limited, however, as we have no direct assessment of food availability, and thus, overall breeding conditions. Because more offspring tended to be female in more urbanized habitat, and the production of female offspring may not be disadvantageous to chickadees under good conditions, we suggest the assumptions of sex allocation theory may not pertain to mountain chickadees.

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3.7. Tables

Table 3.1. Summary of mountain chickadee sex ratio data for the 2014–2016 breeding seasons. Wilcoxon signed-rank tests were performed to determine if population or brood sex ratios deviated from unity. A single case of renesting was excluded from 2016 analyses.

Year	Broods	Nestlings sexed	Population sex ratio	W	p	Mean brood sex ratio \pm SE	W	p
2014	15	82	0.44	-1.10	0.27	0.44 ± 0.04	-1.38	0.17
2015	17	94	0.49	-0.21	0.84	0.51 ± 0.05	-0.02	0.98
2016	13	84	0.40	-1.81	0.07	0.41 ± 0.07	-1.65	0.10

Table 3.2. Results of generalized linear mixed models examining the influence of social male and genetic father condition on nestling sex.

Variable	Estimate	SE	χ^2	<i>p</i>	<i>n</i> (offspring)	<i>n</i> (broods)
Social male condition						
Male age	-0.11	0.30	-0.39	0.70	209	36
Male body condition score	-0.12	0.22	-0.56	0.57	209	36
Offspring paternity	-0.59	0.38	-1.54	0.12	209	36
Genetic father condition						
Male age	-0.37	0.32	-1.15	0.25	181	37
Male body condition score	-0.14	0.23	-0.59	0.56	181	37
Offspring paternity	-1.27	0.80	-1.59	0.11	181	37

Table 3.3. Results of generalized linear mixed models examining the influence of female condition on nestling sex.

Variable	Estimate	SE	χ^2	p	n (offspring)	n (broods)
Female age	-0.18	0.33	-0.56	0.58	223	38
Female body condition score	0.12	0.15	0.79	0.43	223	38
Offspring paternity	-0.39	0.36	-1.08	0.28	223	38

3.8. Figures

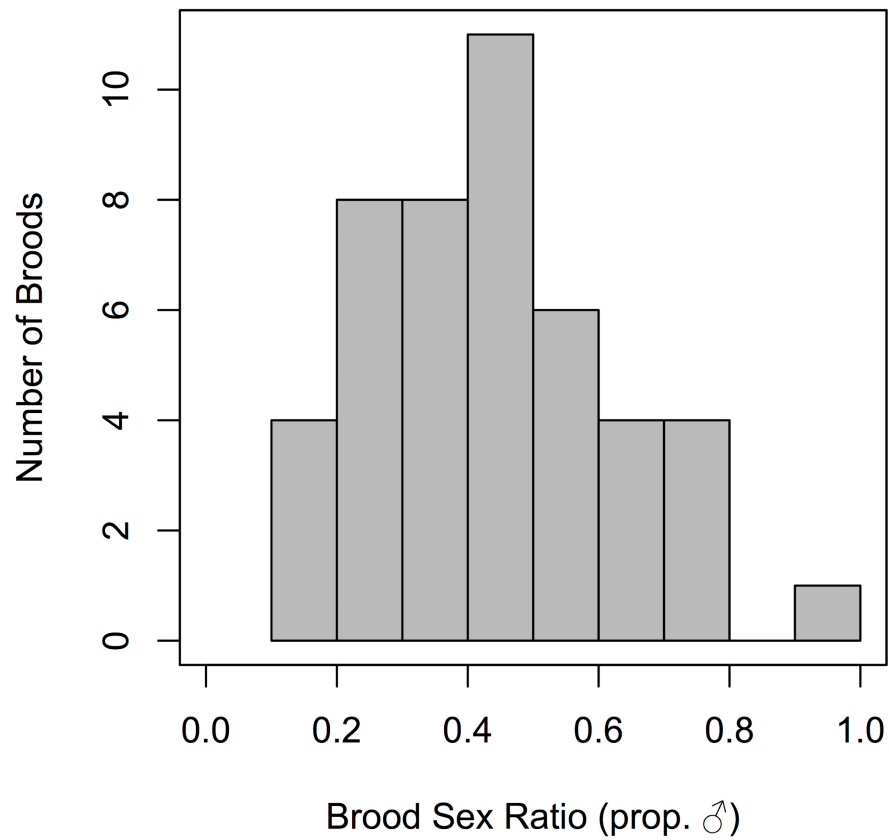


Figure 3.1. Distribution of brood sex ratios for the 2014-2016 breeding seasons. Brood sex ratios ranged from 0.17 (almost exclusively female) to 1.0 (exclusively male).

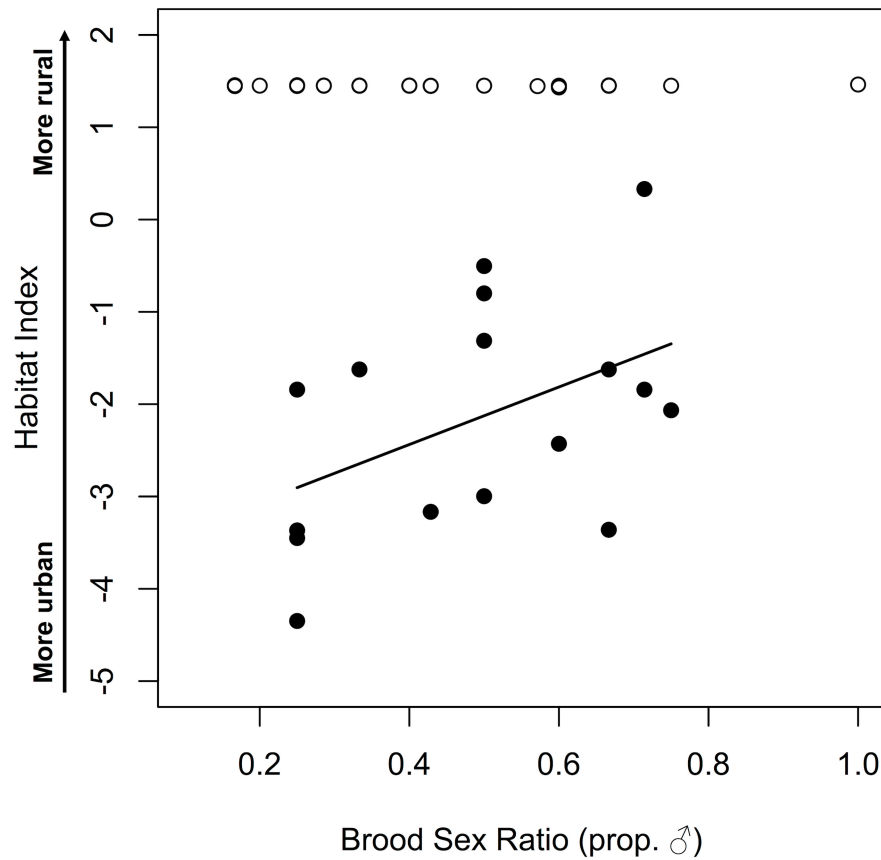


Figure 3.2. Habitat had an effect on nestling sex in nests located in urban (closed circles), but not rural (open circles), habitat. In urban habitat, nestlings were more likely to be female with increasing habitat urbanization (lower habitat index scores).

CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

Variation in individual condition and habitat quality influence reproductive strategies in numerous songbird species (for reviews see Sheldon 1998; Griffith et al. 2002). The goal of this thesis was to gain insight into whether these factors influence reproductive decisions in mountain chickadees. Specifically, I asked how individual condition and habitat urbanization influence the likelihood of a female to engage in EPCs or produce a sex-biased brood. I found evidence to suggest male condition and habitat urbanization influence reproductive decisions in mountain chickadees. I found male, but not female, condition influenced EPP, with older males tending to have a lower proportion of EPO in their nests. In addition, females tended to engage in EPCs with older males. These findings suggest EPP is governed by the good genes hypothesis in mountain chickadees, as has been documented in other Paridae species (e.g., Kempenaers et al. 1992; Otter et al. 1998).

Previous work in my study population classified mountain chickadees as ‘urban adapters,’ as this species is able to successfully inhabit, and appears to benefit from residing in, urbanized habitat (Marini et al. 2017). Although I had predicted EPP to be less frequent in the presumably better-quality urban habitat, I did not find EPP to be influenced by habitat urbanization. I did, however, find offspring sex to be related to the gradient of habitat urbanization. Within urban habitat, offspring from nests in more urbanized habitat were more likely to be female. This finding was surprising because if urban habitat were better-quality habitat, sex allocation theory would predict a male bias. As such, urban habitat may not be as beneficial as originally predicted, or the relative quality of urban vs. rural habitat may be more complex than we had originally predicted. Work in spotted towhees found that, although urbanized edge habitat was comparatively better quality than interior habitat, EPP

rates were highest at the habitat edge and interior, and lowest at intermediate distances. The authors speculated this finding was due to additional factors in the urbanized edge habitat leading to higher EPP rates than expected (e.g., higher densities of transient birds due to feeders drawing in individuals from the surrounding rural habitat) (Smith et al. 2016). This example illustrates how multiple habitat-specific factors can act together to influence reproductive strategies.

Urban habitats typically have significantly different vegetation composition than a species' native habitat (Blair 1996), and this is especially true for conifer-associated species, like the mountain chickadee. Compared to our conifer-dominated rural nest sites, our urban nest sites had lower overall vegetation cover, but greater deciduous cover. Deciduous trees typically bear greater insect abundance and diversity (Southwood 1961), and consequently, urban habitat may be more resource-rich. I constructed a vegetation index to describe the deciduous and coniferous cover and proxy insect availability at each nest site; however, I did not find this index to predict EPP or offspring sex. I conducted this assessment passively (i.e., using satellite images), and consequently, it was lacking information on vegetation species, insect counts, etc. Thus, a more detailed assessment of vegetation composition and insect abundance at nest sites in the field is necessary to accurately describe resource availability and relative habitat qualities.

To date, little research has been conducted on the breeding behaviour of mountain chickadees, and this thesis is the first to investigate sex allocation and within-species EPP in this species. In addition, few studies have considered the impacts of urbanization on mountain chickadees (but see LaZerte et al. 2017; Marini et al. 2017). The findings discussed here provide insight into the natural breeding behaviour of mountain chickadees, and help to further our understanding of the effects of urban settlement on this species. Because my

findings regarding habitat urbanization were opposite to my predictions, I suggest the effects of urbanization on mountain chickadee reproduction may be more nuanced and complex than initially thought. While urban habitat does not seem to negatively impact mountain chickadees, it is plausible the habitat-related differences Marini et al. (2017) observed do not represent benefits per se, but rather suggest urban habitat is neither better nor worse for this species. Nonetheless, further characterization of the two habitat types and their impacts on mountain chickadees is necessary before conclusions regarding relative habitat quality are drawn.

4.1. Future directions

- 1) In order to further decipher the impacts of urbanization on mountain chickadees, it is necessary to quantify how urban and rural habitats differ, and relate these differences to the overall quality of each habitat type. In this study, I relied on a passive assessment of vegetation composition to proxy food availability at each nest site. It is necessary that future studies assess the vegetation composition and insect abundance/ diversity in the field. Prey abundance and diversity could be determined by deploying frass collectors and insect traps along the urbanization gradient. In addition, I suggest situating the cameras for nest watch recordings in such a way that the prey item being provisioned can be identified. When combined with landscape level insect abundance/ diversity data, this information could provide insight into how the insects selected for forage are related to prey availability. A view of prey items could be obtained simply through more strategic placement of the camera outside the box. Alternatively, the addition of an infrared camera inside the nest box could provide a direct view of the prey item, and have the additional benefit of recording adult and nestling behaviour during provisioning trips.

- 2) I found parents provisioned nests more frequently when they contained a greater proportion of EPO; however, whether this finding can be attributed to parental characteristics or habitat quality is unknown. For future nest watch recordings, I suggest situating the camera in a way that individual identity can be determined. I was able to determine individual identity in one recording for which the camera had been attached upside-down to a branch perpendicular to the nest box entrance. This set-up allowed for a close-up view of the attending bird from the underside (thus, colour bands could be viewed). Because most trees did not have branches in such close proximity to the nest box, the construction of an apparatus to attach the camera to the tree in such a fashion would be beneficial. Radio frequency identification could also be used to identify the provisioning individual, and provide higher-resolution provisioning data over longer time periods. As mentioned above, it would be beneficial to situate the camera in a way that prey items could be identified, and relate prey type to nesting success, habitat, or breeding behaviour.
- 3) Finally, the genetic data generated from this study could be applied to questions other than those posed here. For example, the microsatellite data I collected could be applied to the genetic diversity hypothesis of EPP (Griffith et al. 2002). By calculating the heterozygosity of each individual of known paternity, one could ask whether females engage in EPCs to increase the genetic diversity of their young (i.e., are EPO more heterozygous than WPO?). In addition, this inquiry could be expanded to the different habitat types to ask, for example, whether there is lower genetic diversity in one habitat vs. the other.

4.2. Conclusions

In conclusion, this study has demonstrated that: 1) EPP is common in the mountain chickadee and can be attributed to the good genes hypothesis, with older males tending to sire fewer EPO and females engaging in EPCs with adult males; 2) sex allocation appears to occur in this species in response to habitat urbanization, though, whether the assumptions of sex allocation theory hold for mountain chickadees is unclear; and 3) in general, habitat urbanization has little effect on mountain chickadee reproductive tactics, suggesting further characterization of the relative quality of urban vs. rural habitat is necessary. My work has expanded on the findings of Marini et al. (2017), and provided new insight into the reproductive behaviour of mountain chickadees. However, this is only one more piece to the puzzle, and continued study of this population is necessary to disentangle the intricacies of the urban vs. rural habitat dynamic.

4.3. Literature cited

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