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Patterns of extra-pair paternity in mountain chickadees

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Abstract

Extra-pair paternity (EPP) is common in chickadees and often attributed to the good genes hypothesis. Females generally seek dominant males, who are typically larger, older and sing at higher rates than subordinate males, as extra-pair sires. In other songbird species, habitat quality and urbanization have been found to influence EPP. Mountain chickadees commonly inhabit suburban habitat, and previous research on our population has shown urbanization may provide benefits to these adaptable songbirds. Here, we ask how individual condition and urbanization influence rates of EPP in mountain chickadees. Over three breeding seasons, we monitored mountain chickadee nests in urban and rural habitat, and determined parentage by genotyping adults and nestlings at six microsatellite loci. Extra-pair paternity is common in mountain chickadees, with extra-pair offspring (EPO) in 43.2% of nests and accounting for 17.9% of offspring. We found tenuous support for the good genes hypothesis with females tending to engage in EPCs with older males. However, we did not find an influence of male or female condition on the proportion of EPO in a nest. In addition, we did not find a significant effect of habitat on EPP rates, suggesting the impacts of urbanization on mountain chickadee reproduction may not extend to altering extra-pair behaviour.

KEYWORDS

condition, good genes hypothesis, habitat quality, mating strategies, *Poecile gambeli*, urbanization

1 | INTRODUCTION

In birds, social monogamy is widespread, but is often coupled with a mixed-mating strategy that includes extra-pair paternity (EPP). There are several hypotheses as to why a female may choose to seek extra-pair copulations (EPCs), including to insure fertilization of her eggs, increase the genetic diversity of her offspring, or to receive direct benefits, like defence or resources, from the extra-pair (EP) male (Griffith, Owens, & Thuman, 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 (reviewed in Griffith et al., 2002). In Parids, EPP is often explained by the good genes hypothesis: females seek 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*) seek EPCs with older, larger males (Kempenaers, Verheyen, & Dhondt, 1997) and males with brighter ultraviolet-blue plumage (Kempenaers et al., 1992), while female black-capped chickadees (*Poecile atricapillus*) seek EPCs with more dominant males (Mennill, Ramsay, Boag, & Ratcliffe, 2004; Otter, Ratcliffe, & Boag, 1994; Otter, Ratcliffe, Michaud, & Boag, 1998; Smith, 1988).

Chickadee social structure revolves around dominance hierarchies (Ratcliffe, Mennill, & Schubert, 2007). Although much of our understanding of this system comes from black-capped chickadees, mountain chickadees (*P. gambeli*) are known to form linear dominance hierarchies within winter flocks (McCallum, Grundel, & Dahlsten, 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, Chruszcz, & Ratcliffe, 1997).

In black-capped chickadees, dominant individuals gain increased access to resources (Ratcliffe et al., 2007) and are sought by females as both social mates (Otter & Ratcliffe, 1996; Ramsay, Otter, Mennill, Ratcliffe, & Boag, 2000) and EP partners (Otter et al., 1994, 1998). Males signal their status through condition-dependent traits, which may provide females with mechanisms to assess male quality (Otter et al., 1997). Dominant males are typically larger, but leaner, and have greater song output than subordinate males (Dixon, 1965; Grava, Grava, & Otter, 2009; Otter et al., 1997; Ratcliffe et al., 2007; Schubert et al., 2007). 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) (Grava et al., 2009; Otter et al., 1997). 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 by-product of differential resource accessibility.

Habitat urbanization can affect food availability and interspecific interactions, and consequently, may impact a female's likelihood to both seek and encounter EPCs. While some species successfully colonize and thrive in urbanized habitat, how a specific species fares is dependent on multiple factors. Mountain chickadees present a unique opportunity to investigate the effects of urbanization on avian reproduction, as unlike other focal species that have been investigated (Bailly et al., 2016), mountain chickadees are native to coniferous forests (McCallum et al., 1999), rather than deciduous forests. 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 (Blewett & Marzluff, 2005; Marzluff, 1997).

For species native to coniferous forests, the opposite may be true. Although lower total canopy cover, there is greater deciduous vegetation at our 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, as compared to coniferous forest. 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 our study population initiate breeding earlier in urban habitat (Marini, Otter, LaZerte, & Reudink, 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, Peters, & Nowicki, 2004).

Extra-pair paternity has been related to habitat quality in other songbird species. In house sparrows (*Passer domesticus*), experimentally increased food availability resulted in pairs spending more time together at the nest, which, in turn, led to a fivefold reduction in EPP rates (Václav, Hoi, & Blomqvist, 2003). In superb starlings (*Lamprotornis superbus*), Rubenstein (2007) found EPP to be less prevalent in higher quality territories (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, Hoi, Romero-Pujante, & Valera, 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 seek 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, McKay, Murphy, & Duffield, 2016). As such, 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 seek 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 seek EPCs, and males in good condition will be less likely to lose paternity. In addition, 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 high-quality habitat) than rural habitat, although as shown by Smith et al. (2016), urban habitat may promote unexpected breeding strategies.

2 | METHODS

2.1 | Study site

We monitored mountain chickadees breeding in nest boxes in urban and rural areas of Kamloops, BC, Canada during the 2014–2016 breeding seasons (May-Jul). Nest boxes were distributed throughout south Kamloops on a gradient of rural to urban habitat. Rural nest boxes were located in Kenna Cartwright Park, an approximately 8 km^2 wilderness area consisting primarily of Great Basin grassland habitat (e.g., sagebrush, *Artemisia tridentata*; saskatoon, *Amelanchier alnifolia*; Poaceae spp.), but with mature ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga mensiesii*) forests occupying approximately 20% of the park; it is these forests occupied by mountain chickadees. Urban nest boxes were spaced in several clustered patches distributed over approximately 37 km^2 of various urban and suburban areas of south Kamloops, including the Thompson Rivers University campus, neighbourhood parks and backyards of participating citizens. The interspacing of nest boxes within these clustered patches was similar to the spacing with which they were deployed in our rural site. 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).

2.2 | Nest monitoring and sampling

We monitored nest boxes every 1–3 days to identify the breeding pairs occupying boxes and measure breeding success. We caught adults at the nest, either while brooding or feeding, and banded them with a Canadian Wildlife Service (CWS) aluminium 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 (Bonderud et al., 2017). 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, negative residual values may suggest an individual is in better condition.

Six days after hatching, we banded nestlings with a single CWS aluminium 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 | Dawn vocalization recordings

We recorded dawn vocalizations from males breeding in the urban ($n = 9$ unique males) and rural ($n = 9$ unique males) study sites between 4 May and 16 May during the 2014–2016 breeding seasons. We recorded dawn vocalizations using a Sennheiser ME67/K6 microphone and either an Olympus LS-14 or a Marantz PMD670 digital recorder on settings of at least 44 kHz sampling frequency and 16-bit digitization, or higher. We arrived on-site approximately 30 min before sunrise to determine dawn vocalization start time and obtain full recordings. We considered male dawn vocalizations to be finished following a five-minute period of silence following the last vocalization. Dawn vocalization recordings began between 04:34 and 05:21, and lasted an average of 40 ± 13 min (mean ± *SD*, $n = 18$). To ensure the male being recorded was the male associated with a given nest, we began recording at the nest box itself and only recorded within a 75 m radius of the nest box. Often, the vocalizing male was observed copulating with the female at the nest box following cessation of dawn vocalizations, providing further confirmation the recorded male was the male associated with that nest. We analysed recordings using Avisoft–SAS Lab Pro (Avisoft Bioacoustics, 2017) and calculated total vocalization (songs and calls) rate (vocalizations/min) as a measure of male condition.

2.4 | Habitat classification

2.4.1 | Habitat index

Because our study sites varied along a gradient from natural habitat to urban neighbourhoods, we used a habitat index developed by LaZerte, Otter, and Slabbekoorn (2017; scripts available from https://github.com/steffilazerte/urbanization-index) to assess ground cover and measure the degree of habitat urbanization at each nest location. We 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). We imported these aerial images into the image manipulation software GIMP v2.8.16 (The GIMP Team, 2015), where we manually classified the buildings, pavement, natural and non-native grasses, deciduous trees and coniferous trees around each point location. We 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 habitat. The first principal component (PC1) accounted for 68% of the total variation in habitat ground cover. Higher PC1 values corresponded to a greater number of coniferous trees and natural grass cover, and less cover of deciduous trees, non-native grasses and urban features (i.e., greater natural vegetation cover, increasing "rural-ness," decreasing "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).

2.4.2 | Vegetation index

Following methods similar to those used in the habitat index, we assessed only the vegetation at each nest location to determine vegetation type and cover, and proxy food availability. We obtained aerial images of each nest location from Google Earth (Google Inc., 2015), and using GIMP (The GIMP Team, 2015), 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. We conducted a PCA in R (R Core Team, 2016) 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.

2.5 | Molecular methods

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

We assessed nestling parentage by genotyping all adults and nestlings at six avian microsatellite loci (Table 1): Pat14 (Otter et al., 1998), Pat43 (Otter et al., 1998), Titgata02 (Wang, Hsu, Yao, & Li, 2005), Titgata39 (Wang et al., 2005), Escμ4 (Hanotte et al., 1994) and Escμ6 (Hanotte et al., 1994). We amplified DNA in 10 μl reactions containing 1× buffer, 1.5–2.5 mM $MgCl_2$ (1.5 mM: Pat43, Escμ6; 2.0 mM: Pat14, Titgata39; 2.5 mM: Titgata02, Escμ4), 0.25 U *Taq* DNA polymerase, 0.5 μM forward primer, 1.0 μM reverse primer and 0.05 μM fluorescently labelled M13 primer. All forward primers were synthesized with a M13 sequence on the 5′ end to allow for incorporation of the fluorescently labelled M13 primer. We added 1% formamide to reactions involving Pat14 and Escμ4.

We amplified all loci using a two-step annealing protocol: one cycle of 94°C for 2 min, 50°C for 45 s and 72°C for 1 min, followed by seven 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 (Escμ4 and Escμ6), the third step was increased from 25 to 31 cycles. For one locus (Escμ4), 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.

TABLE 1 Allelic variation at the six 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
Escμ4	170–184	8	0.70	0.76
Escμ6	128–152	13	0.91	0.85

2.6 | Parentage assignment

We assigned parentage (both paternity and maternity) first by hand, and then again using CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007). In some cases, we were not able to genotype individuals at all six loci due to insufficient quantities of DNA, which resulted in amplification failure. Previous parentage studies in black-capped chickadees employed only three microsatellite markers but still excluded sires with a high degree of confidence (Mennill et al., 2004; Otter et al., 1998). Thus, we only included nestlings with three or more loci successfully typed in parentage analysis (only one offspring was excluded for not meeting those criteria). We classified offspring as EP if they had two or more mismatches with the putative mother or father (Mennill et al., 2004). We then conducted parentage analysis in CERVUS and combined these results with our manual assignments. In CERVUS, we set 99% strict and 95% relaxed confidence limits. We estimated 75% of the male population had been genotyped based on surveys of the proportion of banded vs. unbanded birds observed in our rural population over the breeding season, and based on similar estimates within the clustered study areas within the urban population. CERVUS did not identify any additional EPO; however, eighteen offspring we 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 ($n = 8$); if not, we retained the manual assignment ($n = 10$). 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 (≥95% confidence) and breeding information from our study population, we created an index of confidence in the realistic validity of the EP sire assignments (i.e., we asked whether it was realistic for the identified male to have encountered the female and sired EPO in her nest). For each EPO, the identified father was only considered a realistic EP sire if he had held territory within 500 m (measured from box-to-box) of the female at some point during the study period. 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. As the average interterritory spacing between territories in our rural study site is approximately 250–300 m, this distance would include males up to two 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).

2.7 | 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, five had an unidentified female, nine had an unidentified male, and one had neither adult identified. In total, seven males and seven 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. Only first broods within a year were included in our analyses.

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 six nestlings from six broods because mortality occurred before collection on day 12. 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, 252 nestlings (rural: $n = 155$, urban: $n = 97$) from 44 broods (rural: $n = 28$, urban: $n = 16$) were included in our analyses.

We conducted statistical analyses in STATA 14 (StataCorp, 2015). 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). To ask whether rates of EPP differed between the study years, we constructed generalized linear mixed models (GLMMs) with logit link functions and binomial error distributions. We included the number of EPO 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 eight females produced more than one brood across the study period, and we assumed EPCs to be sought by females (Otter & Ratcliffe, 1996; Otter et al., 1999; Ramsay et al., 2000; Smith, 1988), we included female identity as a random effect. We did not find the proportion of EPO to differ between 2014 and 2015, or 2014 and 2016; however, we found a greater proportion of EPO in 2016 as compared with 2015 (2014 vs. 2015: $\chi^2 = -0.74$, $p = .46$; 2014 vs. 2016: $\chi^2 = 1.08$, $p = .28$; 2015 vs. 2016: $\chi^2 = 2.12$, $p = .03$).

To ask how male condition predicted the proportion of EPO in his nest, we constructed GLMMs with logit link functions and binomial error distributions. We used the number of EPO in the nest as the response variable, the total number of offspring in the nest as the binomial denominator, age (ASY/SY) and body condition (mass x tarsus regression residual) as predictor variables and included study year and male identity as crossed random effects to account for multiple observations of the same breeding adult across study years, and interannual variation in EPP rates. We included “age x body condition” as an interaction term and dropped it if non-significant ($p > .10$) to derive the final model. We ran a second model using male total vocalization (songs and calls) rate (vocalizations/min) as predictor variables as dawn vocalization data were only available for a subset of males. To ask how female condition predicted the proportion of EPO in her nest, we repeated the above analysis using measures of female condition (mass x tarsus regression residual, age) as the predictor variables, and study year and female identity as crossed random effects. Finally, to ask how habitat influenced the proportion of EPO in a nest, we constructed a similar GLMM using our habitat and vegetation indices as the predictor variables, and study year and female identity as crossed random effects.

2.8 | Ethical note

All work was approved by the University of Northern British Columbia Animal Care and Use Committee and was conducted under a Canadian Federal Master Banding Permit and Scientific Collection Permit no. 22806.

3 | RESULTS

3.1 | General patterns of parentage

In high-density populations, only a small proportion of the total population is typically sampled. However, in both of our study areas, mountain chickadees are found in low densities (E. Bonderud, personal observation). Based on the proportion of banded adult males detected during breeding, we estimated 75% of the males had been sampled in our study. As is typical of many studies, we were only able to sample social males from a subset of the nests ($n = 36$) in which we genotyped offspring ($n = 46$). When conducting manual parentage assignment for the 10 broods with no social male genetic information, we used a conservative approach and assumed that if all nestlings shared a single set of paternal alleles that these were obtained from the social male rather than an extra-pair male. EPP rates were similar when all nests were included (17.9% EPO) and when nests with no sample from the social male were excluded (18.2% EPO).

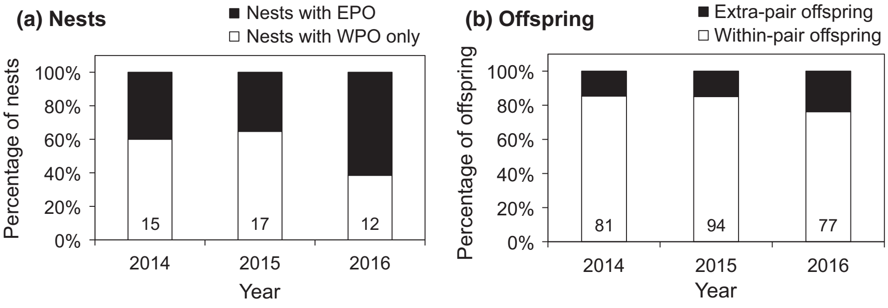

FIGURE 1 (a) Percentage of nests containing at least one extra-pair offspring (EPO) and all within-pair offspring (WPO); (b) Percentage of offspring that were extra-pair and within-pair. Numbers within bars represent total number of (a) nests and (b) offspring sampled in each study year

Overall, 17.9% (45/252) of offspring were sired by an EP male, and 43.2% (19/44) of nests contained EPO (Figure 1). The percentage of EPO within nests with mixed parentage ranged from 12.5% (1/8 offspring EP) to 100% (4/4 offspring EP) in a single nest; the majority of mixed parentage nests (13/19) contained under 50% EPO, with an average 39.8% EPO per nest. Exclusionary power based on the six microsatellite loci was 0.987 for the first parent, 0.999 for the second parent, and 0.999 combined. We were able to confidently identify seven EP males from six (31.6%) 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 four nests, one of which was the nest with two EP males identified, resulting in a total of five social male/EP male pairs for comparison (see below). All offspring were determined to be the genetic offspring of their putative mother.

3.2 | Condition and extra-pair paternity

We did not find either female age ($\chi^2 = -0.15$, $p = .88$) or body condition ($\chi^2 = -0.14$, $p = .89$) to influence the proportion of EPO in her nest (Table 2). Likewise, we did not find any measures of male condition (age, body condition or vocalization rate) to influence the proportion of EPO in the nest (all $p > .13$; Table 2).

3.3 | 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 ($t_4 = 1.38$, $p = .24$) or age ($W = -1.73$, $p = .08$) between the two, but the small sample size ($n = 5$) for these comparisons resulted in low power. In all five social male/EP male pairs, the EP male was of equivalent age (ASY vs. ASY, $n = 2$) or older than the social male (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).

3.4 | Habitat effects

We found neither the habitat index ($\chi^2 = -1.52$, $p = .13$) nor vegetation composition ($\chi^2 = 1.37$, $p = .17$) of nesting habitat to have an effect on the proportion of EPO in a nest (Table 2, Figure 2).

4 | DISCUSSION

Extra-pair paternity has been well-studied in Paridae species, but, until now, intraspecific EPP has 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 (Mennill et al., 2004; Otter et al., 1998; Ramsay et al., 2000). 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, Peters, Johnsen, & Kempenaers, 2007) and great tit (*Parus major*, 40% of nests; Lubjuhn, Gerken, Brün, & Epplen, 1999). In addition, high rates of EPP have been observed in black-capped chickadees hybridizing with Carolina chickadees (*Poecile carolinensis*, 55.6% of nests; Reudink, Mech, & Curry, 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 (Mennill et al., 2004; Otter et al., 1998). In blue tits, several measures of condition appear to influence the decisions of females, with larger males (Kempenaers et al., 1997), older males (Kempenaers et al., 1997), males with greater song output (Kempenaers et al., 1997) and males who begin singing earlier (Poesel, Kunc, Foerster, Johnsen, & Kempenaers, 2006) being sought as EP sires. Here, we asked how male condition, as measured by age, weight relative to body size and dawn vocalization rate influence his likelihood to lose paternity. Contrary to these studies, we did not find a significant relationship between male condition and the proportion of EPO in his nest. Although we had few cases where both the social male and the EP male were known, among those where this information was available, 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, although 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 consistent with the good genes hypothesis.

TABLE 2 Results of generalized linear mixed models asking how female condition (age, mass x tarsus regression residual), male condition (age, mass x tarsus regression residual, vocalization rate) and habitat characteristics (habitat index, vegetation index) influence the proportion of EPO in nests

Variable	Estimate	*SE*	χ^2	*p*	*n* (broods)
Female condition models					
Female age	−0.16	1.06	−0.15	.88	37
Female body condition score	−0.12	0.86	−0.14	.89	37
Male condition models					
Male age	−1.49	0.97	−1.53	.13	35
Male body condition score	−1.00	1.03	−0.97	.33	35
Male vocalization rate (vocalizations/min)	0.06	0.18	0.35	.73	24
Habitat models					
Habitat index	−0.84	0.55	−1.52	.13	44
Vegetation index	0.83	0.60	1.37	.17	44

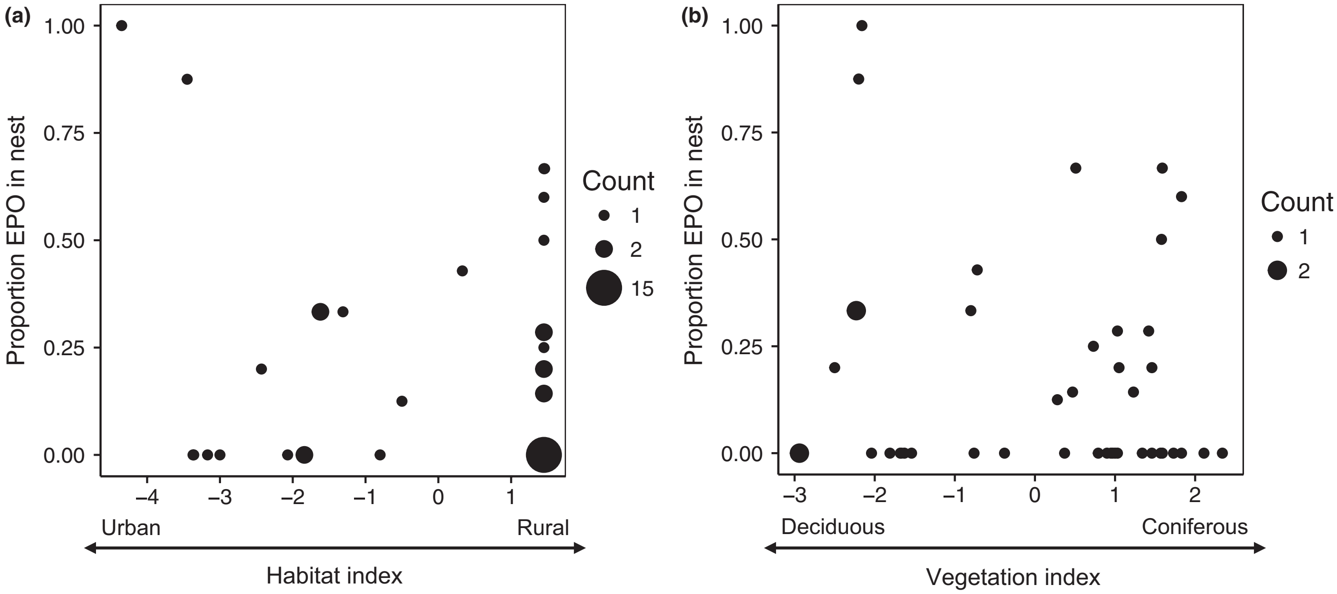

FIGURE 2 The proportion of extra-pair offspring (EPO) in a nest was not influenced by either (a) the habitat index or (b) vegetation index of the nesting habitat. These indices were derived to describe (a) the degree of habitat urbanization and overall quality, and (b) vegetation composition, canopy cover and food availability

In contrast to 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; Rubenstein, 2007; Václav et al., 2003). 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 of slightly better quality than rural habitat to the mountain chickadees in our study population (Marini et al., 2017), possibly 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. In blue tits, Kempenaers, Borgström, Loës, Schlicht, and Valcu (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. In addition, males in lighted territories began singing earlier. 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.

As 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 (Kempenaers 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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CONFLICT OF INTEREST

We declare no conflicts of interest regarding this research.

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