

University of Nevada, Reno

**The causes and consequences of complex behavioral trait variation in a
resident montane bird**

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Ecology, Evolution, and Conservation Biology

By

Benjamin Ross Sonnenberg

Dissertation Advisor: Dr. Vladimir V. Pravosudov

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We recommend that the dissertation
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Advisor

Committee Member

Committee Member

Committee Member

Graduate School Representative

Markus Kemmelmeier, Ph.D., Dean
Graduate School

Abstract

The causes and consequences of complex trait variation are of broad interest in the field of behavioral ecology. In the wild, animals experience wide ranging environmental conditions, and understanding the impact of this heterogeneity in shaping behavioral traits, such as cognition, is critical to understanding their evolution. Though it is thought that environmental conditions contribute to large inter- and intra-species variation in cognitive abilities, the mechanisms generating this variation remain poorly understood. Past laboratory-based work has shown that energetically expensive cognitive traits are impacted by developmental perturbations such as non-optimal developmental conditions (e.g. poor parental care, low-quality nest environment, etc.), but whether these same patterns are exhibited in natural populations is relatively unstudied. This dissertation provides novel insights into the mechanisms shaping complex behavioral trait variation in wild systems focusing on two behavioral traits, spatial cognitive abilities and nest building, in free-living mountain chickadees (*Poecile gambeli*).

Chapter 1 describes the role of directional natural selection acting on spatial cognitive ability using a cohort comparison approach in a wild population. This study showed that spatial cognitive ability does not change within individuals across years, that adult birds on average performed better than first-year birds, and that members of a first-year cohort that performed worse on a spatial cognitive task were less likely to survive than those with better performance. This is some of the first evidence of natural selection acting on a cognitive trait in the wild.

Chapter 2 investigates causes and consequences of variation in nest size in chickadees at different montane elevations with different climatic conditions. This study showed a large degree of temperature variation across all nestling developmental periods and between elevations, but this variation was unrelated to nest size or reproductive output in chickadees. This result contrasts results from studies in other populations of cavity-nesting birds that appear to construct nests corresponding to local environmental conditions. However, I found that female chickadees build highly repeatable sized nests across years. High repeatability in behavioral traits implies a heritable component, suggesting that female nest size is controlled by an innate mechanism.

Chapter 3 considers variation in nestling immune response, ectoparasite load, and nest composition to determine how these developmental conditions shape behavioral trait variation. Ectoparasites are known to have fitness consequences for their hosts, but there is mixed evidence of how widespread and impactful these effects are on the traits of developing avian nestlings. I found that there was no relationship between greater ectoparasite infestation in the nest and offspring mass or immune response, seeming to indicate that this aspect of nestlings' developmental environment may not drive future behavioral trait variation. However, I did find that nest composition—including overall size (i.e., mass), amount of plant materials, and amount of animal materials (i.e., animal hair)—was highly repeatable within individual females, strengthening the evidence that individual variation in nest building behavior is heritable. Identifying

potential post-fledging consequences related to parasite load remains understudied.

Chapter 4 builds upon the work of my earlier chapters by investigating the underlying mechanisms that result in observed chickadee nest repeatability. I found evidence that apparent non-functional trait variation may be related to variation that is under selection through potential shared physiological mechanisms. In this study, I found that food-caching propensity and nest building propensity are not only highly repeatable behaviors within individual female chickadees but also highly correlated. This suggests that food caching, a trait under selection that has a strong underlying drive, may be directly affecting an unrelated behavioral trait, nest building, through a shared mechanism, resulting in a potential behavioral syndrome.

Lastly, chapter 5 presents data testing whether there is a relationship between the physiological burden during offspring development and spatial cognitive abilities, which could explain some of the existing variation in cognitive abilities in wild food-caching mountain chickadees. I used ptilochronology (feather growth rates reflecting nutritional intake) and amount of corticosterone (a hormone associated with broad metabolic processes including stress responses) in feathers ($Cort_f$) of juvenile chickadees to estimate the variation in developmental condition. I found that developmental variation had a limited effect on spatial cognitive abilities, suggesting potential compensatory mechanisms that buffer these specialized cognitive abilities critical for survival in food-caching chickadees from harmful ontological perturbations.

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Table of Contents

Abstract.....	i
Acknowledgments.....	iv
List of Tables.....	vii
List of Figures.....	viii
Background.....	1
Chapter 1: Natural selection and spatial cognition in wild food-caching mountain chickadees – Current Biology, 2019.....	7
Chapter 2: Nest construction, ambient temperature, and reproductive success in a cavity-nesting bird – Animal Behaviour, 2020.....	43
Chapter 3: <i>Protocalliphora</i> prevalence is related to nest composition in a resident cavity-nesting bird.....	90
Chapter 4: Food-hoarding and nest-building propensities are associated in a cavity-nesting bird – Behavioral Ecology and Sociobiology, 2022.....	131
Chapter 5: Natural variation in developmental condition has limited effect on spatial cognition in a wild food-caching bird – Proceedings of the Royal Society B, 2023.....	164
Conclusion.....	215

List of Tables

Chapter 1

Table 1.....	40
Table S1.....	42

Chapter 2

Table 1.....	74
Table 2.....	74
Table S1.....	84
Table S2.....	85
Table S3.....	85

Chapter 3

Table 1.....	125
Table 2.....	125
Table 3.....	125
Table 4.....	126
Table 5.....	127

Chapter 4

Chapter 5

Table 1.....	203
Table 2.....	204
Table S1.....	210
Table S2.....	210
Table S3.....	211
Table S4.....	211
Table S5.....	212
Table S6.....	213

List of Figures

Chapter 1

Fig. 1.....	37
Fig. 2.....	38
Fig. 3.....	39
Fig. S1.....	41
Fig. S2.....	42

Chapter 2

Fig. 1.....	76
Fig. 2.....	77
Fig. 3.....	78
Fig. 4.....	78
Fig. 5.....	79
Fig. 6.....	80
Fig. 7.....	80
Fig. 8.....	81
Fig. 9.....	81
Fig. 10.....	82
Fig. S1.....	87
Fig. S2.....	88
Fig. S3.....	89

Chapter 3

Fig. 1.....	129
Fig. 2.....	130

Chapter 4

Fig. 1.....	160
Fig. 2.....	161
Fig. 3.....	162
Fig. 4.....	163

Chapter 5

Fig. 1.....	206
Fig. 2.....	206
Fig. S1.....	214

Background

Understanding the mechanisms underlying trait variation between and within populations is one of the central goals of ecology and evolution. Populations that span gradients of environmental heterogeneity provide ideal natural experiments to test mechanisms that can limit or generate variation (van Kleunen & Fischer, 2005, De Frenne et al. 2013; López-Goldar & Agrawal, 2021). For example, how organisms respond to environmental challenges across time and space not only informs on the impacts of selection pressures but also about the phenotypic potential of individuals within a population (Endler 1986, Van Kleunen & Fischer, 2005, Trillmich et al. 2015).

Cognitive traits, such as the ability to acquire (i.e., perception & learning), store (i.e., memory), and act on environmental information (i.e., decision-making), are common in a wide range of taxonomic groups (Shettleworth 2009). In fact, there is substantial intra-species variation in cognitive traits across wild populations, and it has long been hypothesized that such trait differences are a product of selection (Pravosudov & Clayton 2002, Shettleworth 2009, Pravosudov & Roth 2013, Boogert et al. 2018). For example, the memory underlying the ability of food-caching animals to recover food items is predicted to be driven by environmental variation such as differences in winter harshness (Vander Wall 1990, Pravosudov & Roth 2013). The successful recovery of food stores (via spatial cognitive abilities) is hypothesized to be critical for their overwinter survival (Pravosudov & Roth, 2013).

In addition to being critical for survival, cognitive traits are also energetically costly (Buchanan et al. 2013, Pravosudov & Roth 2013). As such, another potential driver of variation in spatial cognitive abilities is differences in nutrition-based developmental stress, which has been demonstrated in laboratory studies (e.g., Naninck et al. 2015). Differential responses within taxa inhabiting heterogeneous environments are expected to allow for and generate variation in cognitive abilities (Monaghan, 2008, Buchanan et al. 2013, Trillmich et al. 2015). Behavioral strategies, such as adjustment to onset of breeding time, nest placement and composition, and parental attentiveness, can buffer against the exposure to harmful perturbations and may protect brain development and associated future cognitive abilities of their young (Buchanan et al., 2013).

There is increasing attention devoted to behaviors that show high within-individual repeatability (Brommer & Class 2015, Trillmich et al. 2015). Behavior is traditionally thought of as highly flexible within an individual, but a tendency for behaviors to be highly repeatable implies the opposite (Brommer & Class, 2015, Trillmich et al. 2015). How and why some behavior changes across time thus remains a highly engaging question, especially in wild systems.

My dissertation seeks to understand how individuals cope with environmental uncertainty in montane habitats that present high heterogeneity over a small spatial scale. In addition to investigating the role of spatial cognition in overwinter survival, I aim to understand how individuals, both adult and juvenile animals, cope with annual environmental variation during reproduction and early development. This dissertation provides novel insights into the

mechanisms shaping complex behavioral trait variation in wild systems focusing on free-living mountain chickadees (*Poecile gambeli*).

Mountain chickadees (*Poecile gambeli*) are a food-hoarding resident species inhabiting the montane regions of western North America (McCallum et al. 2020). Here, I studied a population of chickadees that resides across an elevation gradient associated with differences in winter climate severity with increasing elevation in the Sierra Nevada mountains, USA. Chickadees are sedentary and often have very short post-natal dispersal distances after leaving the nest (~1km), making this system ideal for a long-term study (McCallum et al. 2020). I have used novel techniques to measure spatial cognitive abilities in a natural population of mountain chickadees (*Poecile gambeli*), allowing me to pursue several research goals: 1. Investigate the fitness consequences of individual variation in spatial cognitive abilities of wild food-caching birds in their natural environment, 2. Test whether birds' developmental conditions are associated with parental behaviors and subsequent spatial cognitive abilities, 3. Investigate causes and consequences of variation in nest-building.

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Chapter 1

Natural selection and spatial cognition in wild food-caching mountain chickadees

Benjamin R. Sonnenberg¹, Carrie L. Branch^{1,2}, Angela M. Pitera¹, Eli Bridge³, Vladimir V. Pravosudov^{1*}

¹University of Nevada Reno, Department of Biology and Ecology, Evolution, and Conservation Biology Graduate Program, Reno, NV, USA

²Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA

³University of Oklahoma, Oklahoma Biological Survey, Norman, OK, USA

*Corresponding author

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Abstract

Understanding how differences in cognition evolve is one of the critical goals in cognitive ecology. In food-caching species that rely on memory to recover caches, enhanced spatial cognition has been hypothesized to evolve via natural selection, but there has been no direct evidence of natural selection acting on spatial memory. Food-caching mountain chickadees living at harsher, higher elevations, with greater reliance on cached food have better spatial learning abilities and larger hippocampi containing more and larger neurons compared to birds from milder, lower elevations. Here, we tested for natural selection on spatial cognition in wild food-caching mountain chickadees at high elevations and documented the following: (1) compared to first-year juveniles, adults showed significantly better performance on two spatial cognitive tasks—spatial learning and memory and a consecutive reversal learning task; (2) cognitive performance in both spatial learning and reversal learning tasks was not significantly different between years in the same chickadees tested in their first year of life and after surviving to their second winter; and (3) cognitive performance in the spatial learning task was significantly better among the first-year juveniles that survived to their second winter compared to the subset of juveniles that did not survive. Taken together, our results provide evidence for natural selection on spatial cognition in a food-caching species living in harsh environments and suggest that natural selection associated with local environmental conditions might be generating intraspecific differences in cognitive abilities.

Introduction and Results

Cognitive abilities are known to vary both among and within species (Shettleworth 2010, Pravosudov & Roth 2013, Morand-Ferron & Quinn 2015, Morand-Ferron et al. 2016, Morand-Ferron 2017), and it has been hypothesized that both inter- and intra-specific differences in various cognitive traits have evolved via natural selection, with better cognitive performance associated with higher fitness (Pravosudov & Roth 2013). Most evidence of natural selection shaping existing variation in cognition comes indirectly from comparative studies (Krebs et al. 1989, Sherry et al. 1989, Sherry 2006, Shettleworth 2010, Pravosudov & Roth 2013, Morand-Ferron & Quinn 2015, Morand-Ferron et al. 2016, Morand-Ferron 2017), while direct evidence in wild populations remains elusive (Morand-Ferron & Quinn 2015, Morand-Ferron et al. 2016).

Scatter-hoarding species present a convenient model to investigate natural selection on cognition because they store large numbers of food items during autumn, when naturally available food is plentiful, and rely on these caches for overwinter survival (Krebs et al. 1989, Sherry et al. 1989, Sherry 2006, Pravosudov & Roth 2013). The importance of food caches for overwinter survival depends on environmental conditions, with harsher winter conditions associated with greater dependence on food caches for survival (Pravosudov & Roth 2013, Pravosudov & Clayton 2002). It has been well established that most scatter-hoarding species use spatial memory to retrieve stored food (Sherry 2006, Pravosudov & Roth 2013); therefore, individual variation in spatial learning

and memory ability should result in variation in cache retrieval success and potentially survival.

We previously tested this hypothesis by comparing spatial cognition and associated brain morphology among populations of food-caching black-capped chickadees (*Poecile atricapillus*) along both longitudinal and latitudinal gradients of winter climate severity across North America (Pravosudov & Clayton 2002, Roth & Pravosudov 2009, Roth et al. 2010 & 2011 & 2012, Chancellor et al. 2011, Freas et al. 2013) and in mountain chickadees (*P. gambeli*) along an elevational gradient of winter climate severity (Freas et al. 2012 & 2013a,b). In both species, chickadees from harsher environments showed greater food-caching propensity, better spatial learning and memory, and larger hippocampus, a brain region involved in spatial cognition, with significantly more and larger neurons compared to chickadees from milder environments (Pravosudov & Roth 2013). Differences in spatial cognition among populations appear to have a heritable basis, as they persisted in a common garden experiment (Roth et al. 2012) and were associated with differential gene expression in the hippocampus of chickadees reared and maintained in the same controlled environment (Pravosudov et al. 2013). Direct evidence in mammals also suggests that spatial memory and hippocampus volume are heritable (e.g., Croston et al. 2015) and therefore available for selection.

Here, we investigated whether there is natural selection on spatial cognition in wild mountain chickadees at high, harsh elevations in the Sierra Nevada mountains, USA. Despite elevation-related differences in spatial

cognition (Freas et al. 2012, Freas et al. 2013a,b), we found no evidence for genetic population structure between birds from high (harsher winters) and low (milder winters) elevations, suggesting some degree of gene flow (Branch et al. 2017) and even low levels of gene flow can prevent local populations from reaching an equilibrium. Although various evolutionary mechanisms could be at play in the population, we expect directional positive selection to be pervasive because fitness benefits for spatial cognition in a caching species are expected to be especially strong at harsh, high elevations, where reliance on food caches for overwinter survival is greater. However, variation in spatial cognitive abilities at high elevations may be maintained when winters are milder and selection is relaxed.

We developed and implemented a radio frequency identification (RFID)-based system to test spatial cognition in wild, passive integrated transponder (PIT)-tagged chickadees (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). RFID-enabled feeders can be programmed to provide food only to specific individuals while recording the PIT-tag ID and time of each visit by all tagged birds. By assigning each bird to a single rewarding feeder (different feeders for different birds) within an 8-feeder spatial array, we can test spatial learning and memory performance by measuring the number of nonrewarding feeders visited (location errors) prior to landing on the rewarding feeder over multiple trials (a trial starts when a bird visits any feeder in the array and ends with a visit to the rewarding feeder). When a bird visits a rewarding feeder, a door opens allowing access to food, while any other feeder would record the ID and

time of visit without providing food. We followed the same testing protocol for the last two years (Tello-Ramos et al. 2018), which allowed direct comparisons of (a) cognitive performance between individuals in the same cohort of first-year birds who survived their first winter and those that did not and (b) cognitive performance between age classes – adults versus first-year birds.

We employed three well-established methods to detect natural selection. First, if selection on a particular trait occurs at a certain age, the trait frequency distribution can be expected to differ between age classes (age class comparison) (Endler 1986). In mountain chickadees, the largest overwinter mortality occurs during their first winter, so selection on survival-associated traits should be evident between first-year birds and adults. Thus, if there is selection on spatial cognition, we can expect differences in cognitive performance between first-year and adult chickadees (Figure 1A).

Second, when using age class comparison, it is necessary to establish that the trait itself does not change due to development or experience (e.g., learning) during the first two years of life. This is especially critical when evaluating cognitive traits due to their intrinsic flexibility. Therefore, we directly compared cognitive performance of the same birds that were tested as first-year juveniles and the following year as adults (Figure 1B).

Lastly, we documented survival based on individual variation in cognitive performance. We specifically compared cognitive performance of first-year birds that survived until the next year to that of birds from the same cohort that tested at the same time but did not survive (Figure 1C).

These comparisons were conceived to reveal the presence of selection on two spatial cognition metrics—spatial learning and memory ability and reversal spatial learning and memory ability (when each bird was re-assigned to a new and different rewarding feeder immediately following the spatial learning task) (Croston et al. 2017, Tello-Ramos et al. 2018). Spatial learning and memory ability are expected to reflect the ability to remember and recover previously made food caches (Sherry 2006, Pravosudov & Roth 2013), while reversal learning ability is expected to reflect learning and memory flexibility, which may be important for foraging success in unpredictable environments (Tello-Ramos et al. 2018 & 2019). We used mean number of location errors per trial over the first 20 trials (initial learning and memory phase) and the mean number of location errors per trial over the entire 4-day task (overall task performance measuring learning and longer-term memory) (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). As birds learn, they are expected to make fewer and fewer location errors with each trial; thus, the mean number of location errors per trial over the entire task is a reliable and accurate estimate of learning and memory that is repeatable across years within individuals (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). Faster learning and better memory are indicated by fewer location errors per trial, so lower mean number of location errors per trial over the entire learning task indicates better learning and memory. We used the total number of trials completed during each cognitive task as a covariate in all analyses of cognitive performance to control for individual variation in timing and frequency of trials, which allows testing whether groups differed in cognitive

performance independently of potential differences in number of trials (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018).

Spatial Learning Task

Adults significantly outperformed first-year juvenile chickadees during the same season in the spatial cognitive task (GLM, mean number of location errors per trial over the entire task: $F_{1,70} = 17.41$, $p < 0.0001$; total number of trials as a covariate: $F_{1,70} = 30.36$, $p < 0.0001$; effect size: Glass's $\Delta = 1.06$; Figure 2A), and cognitive performance over the entire task was significantly associated with age class (logistic regression: $\beta = -6.27$, $\text{Wald} = 10.24$, $p = 0.001$). Differences between juveniles and adults were also present within the first 20 trials, when birds showed significant improvement between the first 3, 5, 10 and 20 trials with mean number of location errors decreasing with number of trials (Figure 3A). Chickadees continued improving their performance after the first 20 trials as the mean number of location errors per trial over the entire spatial task was significantly smaller than the mean number of location errors per trial during the first 20 trials of the task (repeated-measures GLM, $F_{1,70} = 5.59$, $p = 0.02$; age effect: $F_{1,70} = 11.44$, $p = 0.001$; Figure S1).

There were no significant differences in performance between years in the same cohort of chickadees that were tested in 2016–2017 as first-year juveniles and 2017–2018 as adults (mean number of location errors per trial over the entire task: repeated-measures GLM, $F_{1,33} = 0.28$, $p = 0.59$; total number of trials as a covariate: $F_{1,33} = 6.01$, $p = 0.02$).

Within the 2016–2017 first-year chickadee cohort (e.g., juveniles), individuals that survived and were tested again as adults ($n = 37$) in 2017–2018 performed significantly better in the spatial learning task than members of their first-year cohort that did not survive ($n = 25$) after their first year of testing (mean number of location errors per trial over the entire task: GLM, $F_{1,59} = 4.60$, $p = 0.036$; total number of trials as a covariate: $F_{1,59} = 34.4$, $p < 0.001$; effect size: Glass's $\Delta = 0.6$, Figure 2B). Cognitive performance in the spatial learning task was a significant predictor of survival in first-year chickadees tested in the 2016–2017 season (mean number of location errors per trial over the entire task: logistic regression: $\beta = -2.24 \pm 0.78$, Wald = -3.77 , $p = 0.003$). Significant differences in performance between the juveniles that survived until the next year and ones that did not were detected in the first 20 trials of the spatial learning task which represents the initial learning and memory phase (GLM, mean number of location errors per trial: $F_{1,59} = 11.3$, $p = 0.001$; total number of trials as a covariate: $F_{1,59} = 0.001$, $p = 0.94$; effect size: Glass's $\Delta = 0.88$; Figure 2C). Performance during the first 20 trials of the spatial learning task was also a significant predictor of survival (logistic regression: $\beta = -2.42$, Wald = 8.22 , $p = 0.004$). Differences in performance between survivors and birds that later died were also present within the first 20 trials, when birds showed significant improvement between the first 3, 5, 10, and 20 trials, with the mean number of location errors decreasing with the number of trials (Figure 3B). Chickadees continued to improve their performance beyond the first 20 trials, as the mean number of location errors per trial over the entire task was significantly lower than

the mean number of location errors per trial during the first 20 trials (repeated-measures GLM, $F_{1,59} = 21.3$, $p < 0.0001$, survived versus died: $F_{1,59} = 10.46$, $p = 0.001$; Figure S2).

Reversal Spatial Learning Task

Adults significantly outperformed first-year birds in the reversal spatial learning task (mean number of location errors per trial over the entire task: GLM, $F_{1,61} = 6.91$, $p = 0.01$; total number of trials as covariate: $F_{1,61} = 44.76$, $p < 0.001$; effect size: Glass's $\Delta = 0.74$, Figure 2D), and there was a significant association between the mean number of location errors per trial over the entire reversal task and age class (e.g., adult versus juvenile; logistic regression: $\beta = -8.75$, $\text{Wald} = 5.95$, $p = 0.015$). Chickadees also improved their performance throughout the task as the mean number of location errors per trial over the entire task was significantly lower than the mean number of location errors per trial during the first 20 trials (repeated-measures GLM, $F_{1,61} = 7.28$, $p = 0.009$). Reversal task performance remained the same from the first year to adulthood within the same cohort of chickadees that were tested as first-year juveniles in 2016–2017 and as adults in 2017–2018 (mean number of location errors per trial over the entire task: repeated-measures GLM, $F_{1,28} = 0.87$, $p = 0.36$; total number of trials as a covariate: $F_{1,28} = 2.54$, $p = 0.122$).

There were, however, no significant differences in reversal spatial task performance between chickadees that were tested in 2016–2017 as first-year juveniles and survived until the next year and those juveniles that tested in 2016–2017 but did not survive (mean number of location errors per trial over the entire

task; $F_{1,57} = 0.78$, $p = 0.38$; total number of trials as a covariate: $F_{1,57} = 30.9$, $p < 0.001$). All birds improved their performance throughout the task, as the mean number of location errors per trial over the entire task was significantly lower than the mean number of location errors per trial during the first 20 trials (repeated-measures GLM, $F_{1,57} = 26.3$, $p < 0.0001$).

There were no significant differences in the total number of trials completed during the spatial learning task between first-year birds and adults (Table 1). During the reversal spatial learning task, first-year birds completed significantly more trials than adults (Table 1).

Discussion

Our results provide evidence for natural selection on spatial cognition in wild food-caching mountain chickadees. The strongest support comes from spatial learning and memory performance, as results from all three comparisons are consistent with natural selection: (1) adults showed better spatial learning and memory performance than first-year juveniles; (2) there were no significant differences in performance of the same cohort of chickadees that were tested as first-year birds and then as adults, i.e., they did not improve performance with experience; and (3) spatial cognitive performance was a significant predictor of survival in first-year juvenile chickadees tested in 2016–2017; birds that survived until the 2017–2018 season showed significantly better performance in the spatial learning and memory task compared to birds that died.

We also found large age-class differences in reversal spatial learning and memory performance between adults and first-year chickadees. However, we

detected no significant differences in performance in the reversal task between the subset of first-year birds that tested in 2016–2017 and survived until 2017–2018 and the subset that presumably died. It is unclear why we detected age-class differences, but not differences in survival based on reversal learning performance. One explanation is that chickadees improve performance with age, but there were no significant differences in performance in the same cohort of birds over the two years. Age class comparison is based on random sampling of different age classes and expected to be independent of survival detection accuracy. Considering that > 95% of all birds detected around our arrays (both visually and via RFID feeders) are PIT-tagged and almost all detected PIT-tagged birds come to our RFID feeders, we do not think there was a strong bias in our sampling. Nonetheless, it would be important to document direct fitness consequences of variation in reversal learning performance. It is not entirely clear how reversal learning ability is associated with fitness. Reversal learning is a measure of learning flexibility (Anderson & Neely 1996, Strang & Sherry 2014, Tello-Ramos et al 2018), so it is not likely involved in food caching and retrieval processes. However, learning flexibility is likely important for foraging in unpredictable and changing environments (Croston et al. 2017, Tello-Ramos et al. 2018 & 2019).

It is unlikely that our results were driven by potential differences in motivation as we controlled for the frequency of visits. Additionally, the total number of trials did not differ significantly between first-year birds and adults in the spatial learning task and all birds received the same food reward during each

trial. Interestingly, juveniles tended to complete more trials during each task, but adults still showed better performance.

Our data also suggest that it is unlikely that social/behavioral factors contributed to the observed differences. Juveniles are socially subordinate to adults (Ekman 1989, McCallum et al. 1999), therefore, if social interactions were an important factor, we would expect an improvement in performance with age. We, however, detected no significant differences in performance in the same individuals first tested as juveniles and then the next year as adults suggesting that our measured performance represents an individual's cognitive ability.

When environmental conditions favor particular heritable traits, natural selection can be expected to result in changes in their frequency distribution within a population, potentially leading to reduced variation and even fixation of a trait (Endler 1986). Such results, however, can only be expected in populations lacking new sources of variation (without immigration). In continuously distributed species with no geographic barriers, natural selection across a heterogeneous environment may be essential to maintaining local adaptations in the presence of gene flow (Gonzalo-Turpin & Hazard 2009, McCracken et al. 2009, de León et al., 2010, Oliveira et al. 2015, Tigano & Friesen 2016). In our chickadee system, we previously detected significant differences in spatial cognition and hippocampus morphology (Freas et al. 2012), yet we found no evidence for population genetic structure suggesting gene flow across elevations (Branch et al. 2017).

There is large annual variation in severity and longevity of winter conditions at our study site, therefore, strength of natural selection can be expected to vary among years (e.g., Kozlovsky et al. 2018). Years with milder, drier winters may be associated with relaxed selection on cognitive ability and, in combination with ongoing gene flow, may result in increased variation in spatial cognition at higher elevations. Alternatively, years with severe winters, such as the 2016-17 season which had the highest snow levels in almost 100 years, may be expected to reduce such variation via natural selection (e.g., Kozlovsky et al. 2018).

Several other studies investigated fitness consequences of individual variation in cognitive abilities but focused on novel problem-solving and reproduction (Cole et al. 2012, Cauchard et al. 2013). It is not entirely clear how novel problem-solving ability is associated with fitness, whether it is heritable or whether there are population-level differences. We, however, measured spatial learning and memory, which are well known to be involved in cache retrieval (Krebs et al. 1989, Sherry et al. 1989, Pravosudov & Roth 2013) and previously documented population differences in spatial learning and memory associated with variation in winter climate (Pravosudov & Roth 2013, Freas et al. 2012 & 2013a). In addition, there is some evidence that spatial learning and memory ability is heritable (Roth et al. 2010 & 2012, Pravosudov et al. 2013, Croston et al. 2015).

To our knowledge, this is the first study providing evidence for natural selection on spatial cognition. Our results support our previous comparative

studies showing elevation-related differences in memory and hippocampus morphology over a small spatial scale and suggest that such differences represent local adaptations maintained by natural selection.

Methods

Study subjects and site

We tested spatial cognition performance of wild mountain chickadees (*Poecile gambeli*) fitted with unique PIT-tag identifiers (IB Technology, Leicestershire, U.K). We have been trapping and banding chickadees using mist nets at multiple feeder locations throughout the non-breeding season as well as in nest boxes during breeding since 2014 at our long-term high elevation site (ca. 2400 m; Croston et al. 2017, Tello-Ramos et al. 2018) in Sagehen Experimental Research Forest, near Truckee, CA, U.S.A. All trapped birds were banded with a unique combination of color bands and a PIT-tag. Due numerous issues, birds at our low elevation site (ca. 1900 m) were not tested in the 2017-2018 season. We used data from Tello-Ramos et al. 2018 to assess within individual variation in performance across two testing years. The performance of first-year birds that tested during the 2016-2017 season and were detected in the 2017-2018 season, were compared to birds that tested in the 2016-2017 season, but did not reappear during the 2017-2018 season, and were therefore presumed dead. All testing methods in this study were identical to those in Tello-Ramos et al. 2018. Even though we have been banding chickadees since 2014, only the years included in this study allowed us to compare full cohorts of chickadees with known age.

This research has followed the guidelines of the Institutional Animal Care and Use Committee of the University of Nevada, Reno (Protocol No. 00603), as well as local and federal guidelines (California Department of Fish and Wildlife Permit D-0011776516- 4). As this was a field study, animals were banded and immediately released. We did not detect any detrimental effects of tagging and only collected blood samples when environmental conditions were favorable.

'Smart' feeders and spatial arrays

All spatial memory tasks took place at two different feeder arrays separated by ca. 1.2 km, at the same high elevation locations as in previous studies (Freas et al. 2012 & 2013a, Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). Each spatial array consisted of eight identical RFID-enabled smart feeders mounted on a square aluminum square frame (122 × 122 cm) equidistant from each other with two feeders on each side. Both arrays were suspended from the four nearby trees up to 4 m above the ground to prevent access by bears and rodents. Both arrays were surrounded by trees on all sides and we showed no significant differences in performance between the arrays Croston et al. 2016. Arrays were only lowered to refill the feeders with seed, collect data, and replace batteries. Each feeder was constructed from PVC panels that housed both a hopper, which contained the black oil sunflower seeds, and a bottom compartment that held RFID circuitry, a lithium-ion battery (12 V, 6800 mAh), and a motor that raised and lowered a rectangular door to restrict and allow access to seeds. An antenna connected to the RFID board was imbedded into a perch in front of the feeder opening. The feeders and antennae

were custom made according to the designs by E.S.B and V.V.P (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018), and the RFID electronics followed the design in Bridge and Bonter (Bridge & Bonter 2011). Feeders were programmed to be active from 0600 hours to 1930 hours and when active, feeders recorded PIT-tag IDs, date, and time of visits from all PIT-tagged birds that landed on the perch. Feeder batteries were changed every third or fourth day during testing.

During testing, feeders operated in one of the three feeding modes: open, all, or target. Feeders recorded all visits by any PIT-tagged bird, regardless of the feeding mode in which it was operating. During the pre-testing phase, the feeders were first kept in 'open' mode, where feeder doors remained in the open position and all birds were allowed access to all feeders. Feeders were then set to 'all' mode for one week, in which feeder doors were closed but would open when any PIT-tagged bird landed on the antenna (perch). This allowed birds to habituate to the opening and closing mechanism of the feeder doors. 'Target' mode was initiated at the start of memory testing, where each feeder would only open for birds whose PIT-tag IDs matched a list of IDs that were programmed into the RFID circuit board before testing (different lists of IDs for each feeder). In 'target' mode, all PIT-tagged birds were recorded at all feeders regardless of feeder assignment to track the order and frequency of visits to unrewarding feeders (location errors) before visiting the rewarding (assigned) feeder. We measured spatial learning and memory by using the number of unrewarding (or unassigned) feeders visited prior to visiting the assigned feeder that provided food. The data were divided into trials, wherein each trial began when a bird

visited any feeder in the array and ended with a visit to the rewarding feeder (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). As birds learn, they are expected to make fewer and fewer location errors with each trial and so fewer mean number of location errors per trial averaged over multiple trials is indicative of better learning and memory. Using this system, we previously demonstrated that chickadees learn their assigned spatial feeder by showing a significant reduction in the mean number of location errors per trial with the number of trials (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018) and significantly lower mean number of location errors per trial averaged over the entire task compared to the expected number of errors based on random search (4 errors). Our previous work suggested that using mean number of location errors per trial over the entire task controlled statistically for the total number of trials is a very accurate and reliable measure of learning and memory performance (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). We have also previously shown that chickadees use spatial learning to identify feeder locations, as they return to their previously assigned location, rather than to a specific feeder after the array has been rotated in space (Croston et al. 2016).

Spatial learning task

For the spatial learning task, which directly followed the pretesting phase, all feeders were set to 'target' mode from 30 March, 2018 to 4 April, 2018. Each bird was assigned only a single feeder ('target' feeder) within an array. Assignments were made by first inspecting feeder visit patterns during the pretesting period, and for each bird visiting the arrays, we assigned the least

frequently visited feeder. We also adjusted assignments to ensure that surviving individuals were assigned to different feeders than their assignments in previous years, and that birds that were trapped together were assigned to different feeders.

Reversal learning task

Immediately following the spatial learning task, we conducted a reversal spatial learning task from 4 April to 9 April, 2018, which tests for cognitive flexibility. During the reversal task each bird was re-assigned to a different 'target' feeder. All birds previously assigned to the same 'target' feeder in the initial learning task, were reassigned to different feeders to avoid social learning. In addition, new, reassigned feeders were chosen so that they were on a different side of the array than the previously assigned target feeder (detailed methods in Croston et al. 2017 and Tello-Ramos et al. 2018). Chickadees were expected to stop visiting previously rewarded feeders and learn the location of their newly assigned feeder (Tello-Ramos et al. 2018).

Quantification and Statistical Analysis

Measuring survival

All food-caching Parids, such as mountain chickadees, are permanent residents that have one post-natal dispersal movement during late summer-early fall in their first year of life (Ekman 1989, McCallum et al. 1999). Such sedentary behavior supports the assumption that any birds banded after their settlement, but not detected later have died, which has been used in all studies of *Poecile* species. In our study system, we have banded more than 1000

chickadees (ca. 500 birds at high elevation, ca. 600 at low elevation) with unique PIT-tags during annual trapping since 2014. Recaptures during annual trapping, combined with detection of PIT-tagged birds at our RFID feeders, were used to detect surviving birds (> 95% annual detection rate of living birds). We have never detected an individual moving from post-natal settlement locations; individuals either continue to be detected around the same trapping/breeding sites or disappear completely. Finally, we showed that birds trapped for behavioral lab experiments in the fall and released after spending months in captivity, returned to their original trapping locations (Branch et al. 2016). Therefore, our data are consistent with the general assumption that any chickadee disappearing from our monitored sites between years is likely deceased.

Analyses

For all analyses of spatial cognitive performance, we used the mean number of location errors per trial over the entire learning task and the mean number of location errors per trial made during the first 20 trials of each learning task as dependent variables. Mean number of location errors per trial during the first 20 trials represents initial learning phase that involves learning and short-term memory (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). The overall task performance was defined by the mean number of location errors per trial over the entire task, which likely relies on longer-term memory since birds need to use memory to find the feeder once they learned its location. Smaller mean number of location errors per trial indicate fewer errors on average over multiple

trials during the entire 4-day task. We chose the first 20 trials to analyze the initial learning and memory phase because our previous data showed significant differences between birds using this metric (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). The overall task performance over the entire task is more reflective of a longer-term memory and hence represents a different, albeit complimentary information.

We used the total number of trials completed during each task as a covariate to control for potential individual differences in the number and timing of trials (Croston et al. 2017, Tello-Ramos et al. 2018) and age (adult versus first year) and survival outcome (survived versus died) as independent variables. Total number of trials is negatively associated with the number of location errors per trial due to learning, so using it as a covariate allows direct comparison of performance between groups of interest independently of any potential differences in the number of trials completed (Croston et al. 2016 & 2017, Tello-Ramos et al. 2018). We used general linear models and logistic regressions for statistical analyses in Statistica v. 13 (TIBCO Software Inc.). Inclusion of an array as a random variable had no effect on the results and so it was dropped from the analyses. In all tests, n is the number of birds tested and Figures present either raw data or means and SEM. Significance was defined as P value < 0.05 . We estimated effect sizes for significant results using Glass's $\Delta = (\text{mean } 1 - \text{mean } 2) / \text{SD} 2$. See Table S1 for sample sizes.

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Author Contributions

V.V.P. conceived and designed the study; B.R.S., C.L.B., and V.V.P. collected the data; E.B. designed RFID boards, wrote the software for the RFID boards, and participated in all RFID-related troubleshooting; E.B. and V.V.P. co-designed the RFID-based feeders used in the study; A.M.P. participated in data collection and processed and organized the data using custom Perl scripts; B.R.S. and V.V.P. analyzed the data and wrote the first draft; and A.M.P., C.L.B., and E.B. co-wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

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Figure legends

Fig. 1 Diagram of the comparisons used in this study: (A-C) (A) comparing adults and first-year juveniles; (B) comparing the same individuals which were first tested as first-year juveniles and then next year as surviving adults; (C) comparing individuals that survived until next year and the ones that died within the same cohort of first-year juveniles.

Fig. 2 Performance in Spatial Learning and Memory Task and in Spatial Reversal Learning Task in Juveniles and Adults and in Juvenile Chickadees that Survived and Those that Died: (A) Spatial learning and memory task—class comparison within the same season: mean number of location errors per trial and the total number of trials during the entire spatial learning task in the first-year birds and in adults during 2017–2018 season. See also Figure S1. (B) Spatial learning and

memory task—survival comparison: mean number of location errors per trial and the total number of trials during the entire spatial learning task in first-year birds that either survived or died after the 2016–2017 testing season. See also Figure S2. (C) Spatial learning and memory task—survival comparison: mean number of location errors per trial over the first 20 trials and the total number of trials during the entire spatial learning task in first-year birds that either survived or died after the 2016–2017 testing season. See also Figure S2. (D) Reversal spatial learning and memory task—age-class comparison: mean number of location errors per trial and the total number of trials over the entire reversal spatial learning task in first-year, juvenile birds and in adults during 2017–2018 season. Shaded areas in all graphs are 95% CI.

Fig. 3 Spatial Learning and Memory Performance within the First 20 Trials in Adult and Juvenile Chickadees and in Chickadees that Survived and Those that Died:

(A) Mean number of location errors (least-squares means and SE) per trial during the first 3, 5, 10, and 20 trials of the spatial learning task: comparing juveniles and adults within the same season (repeated-measures GLM, age: $F_{1,71} = 4.61$, $p = 0.03$; trials: $F_{3,213} = 92.2$, $p < 0.0001$). (B) Spatial learning and memory task—mean number of location errors per trial (least-squares means and SE) during the first 3, 5, 10, and 20 trials of the spatial learning task: comparing juveniles that survived until next year with the ones that died (repeated-measures GLM, survived versus died: $F_{1,59} = 12.02$, $p < 0.001$).

Fig. 1

Spatial cognitive performance

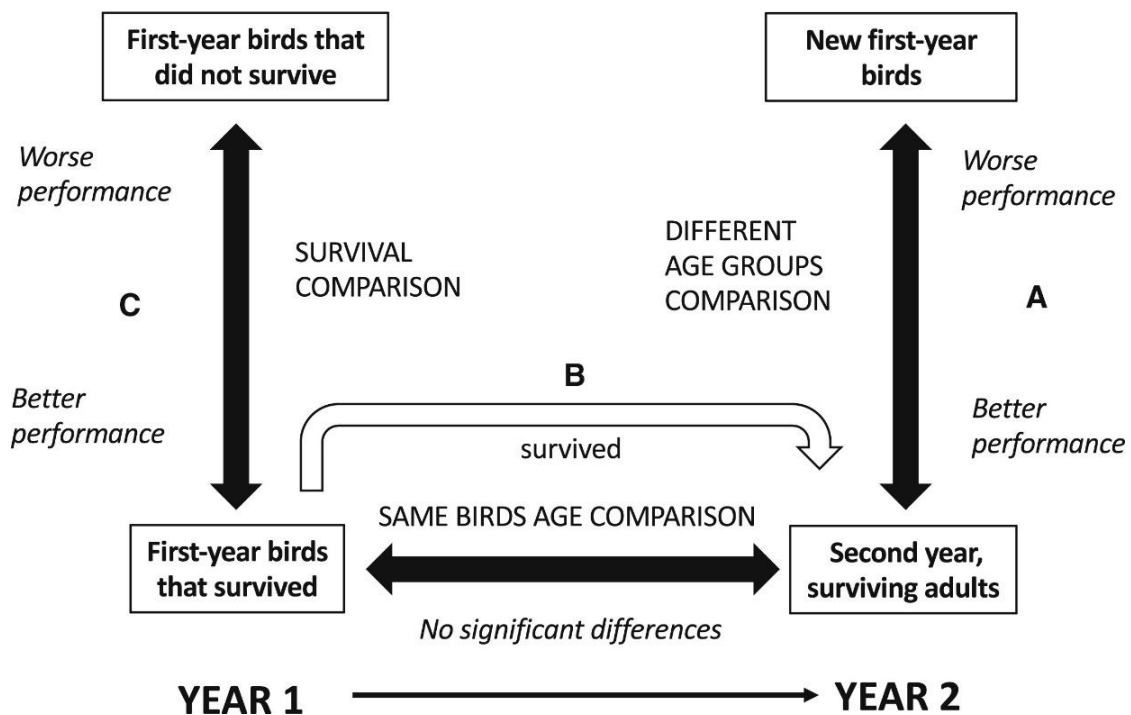


Fig. 2

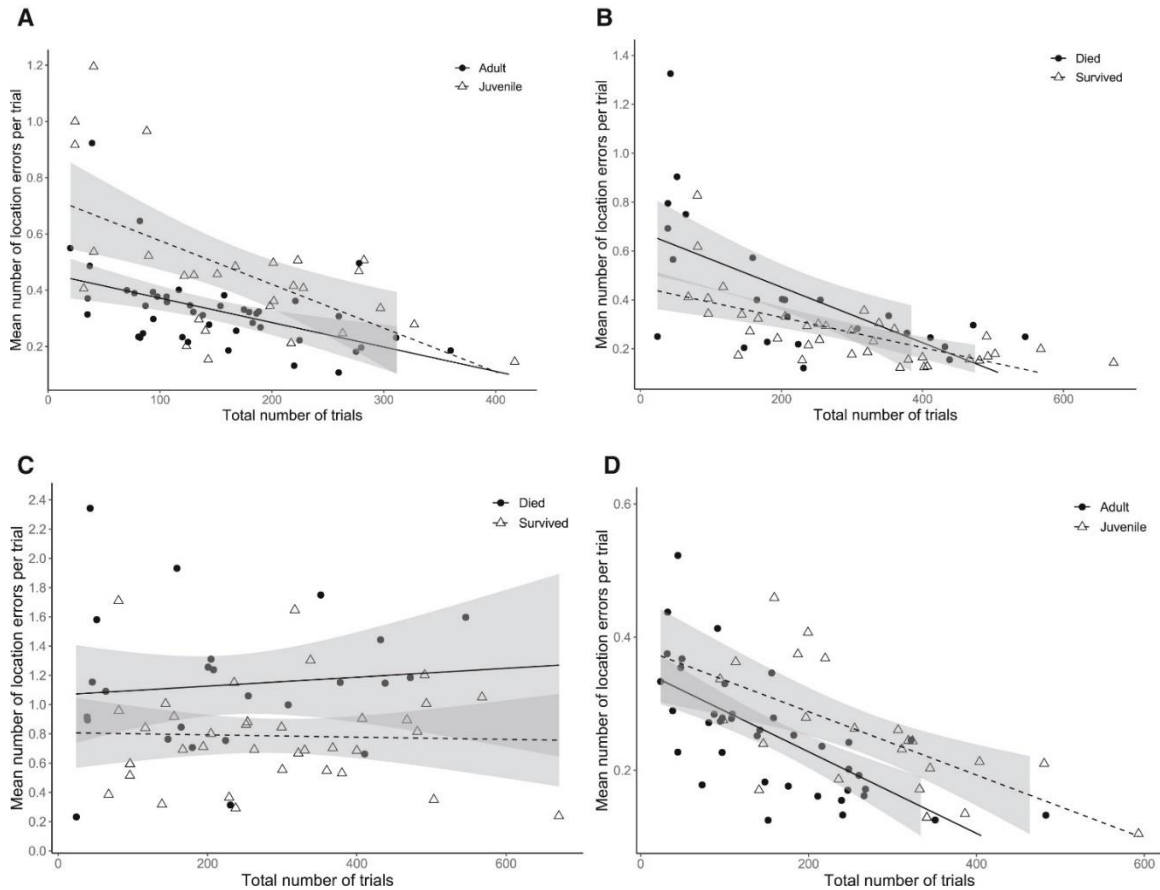


Fig. 3

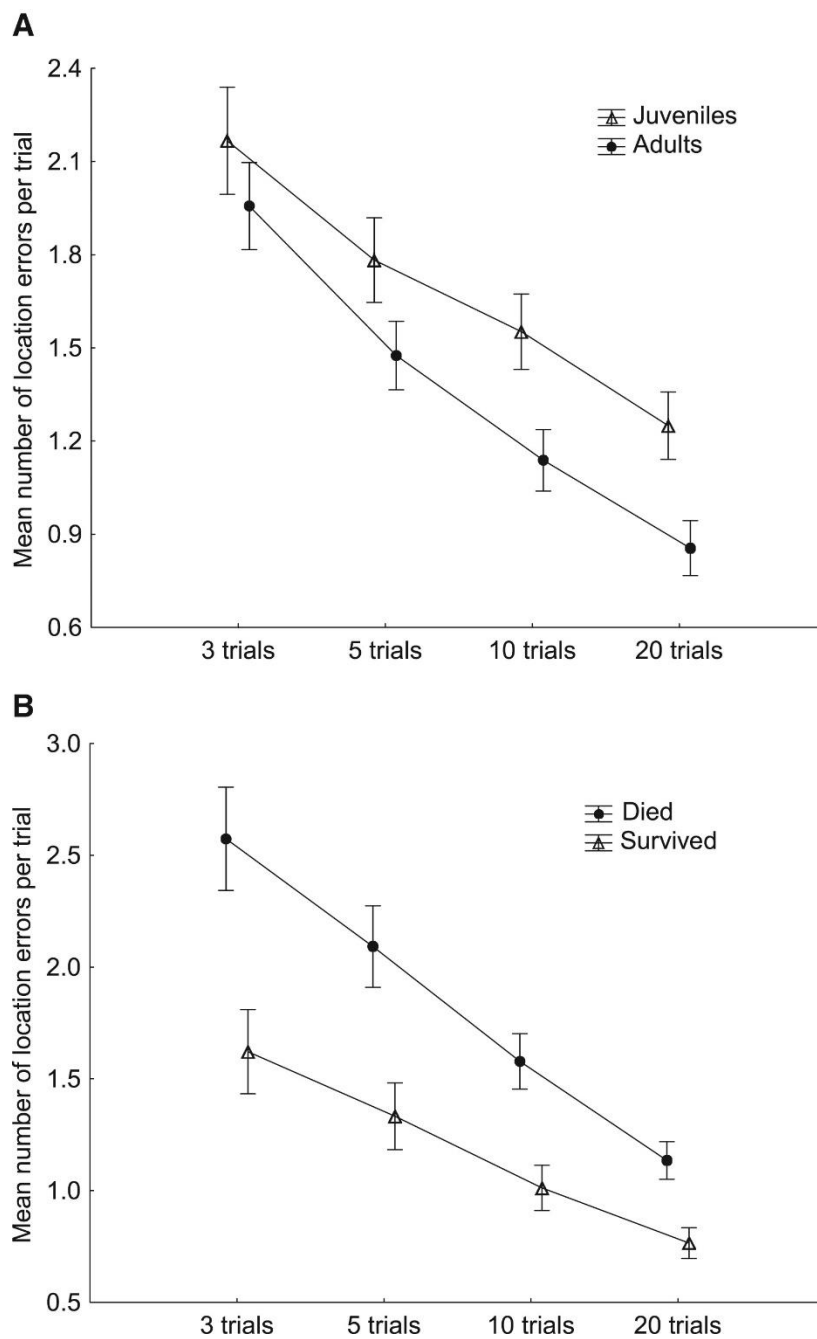


Table 1

Table 1. Total Number of Trials Completed during Each Spatial Cognitive Task during 2017–2018 Season

	First-year birds	Adults	GLM
Spatial learning	171.6 ± 16.7	150.7 ± 13.2	$F_{1,71} = 0.96, P = 0.33$
Reversal learning	269.1 ± 23.7	157.3 ± 17.8	$F_{1,62} = 14.23; P < 0.001$

Supplemental Figures and Tables

Fig. S1 Mean number of location errors per trial (Least Squares Means and SE) during the first 20 trials vs mean number of location errors per trial during the entire spatial learning task in juveniles and adults in the same season. Related to Figure 2A.

Fig. S2 Mean number of location errors per trial (Least Squares Means and SE) during the first 20 trials vs mean number of location errors per trial during the entire spatial learning task in juveniles that survived until next year and those that died. Related to Figures 2B and 2C.

Fig. S1

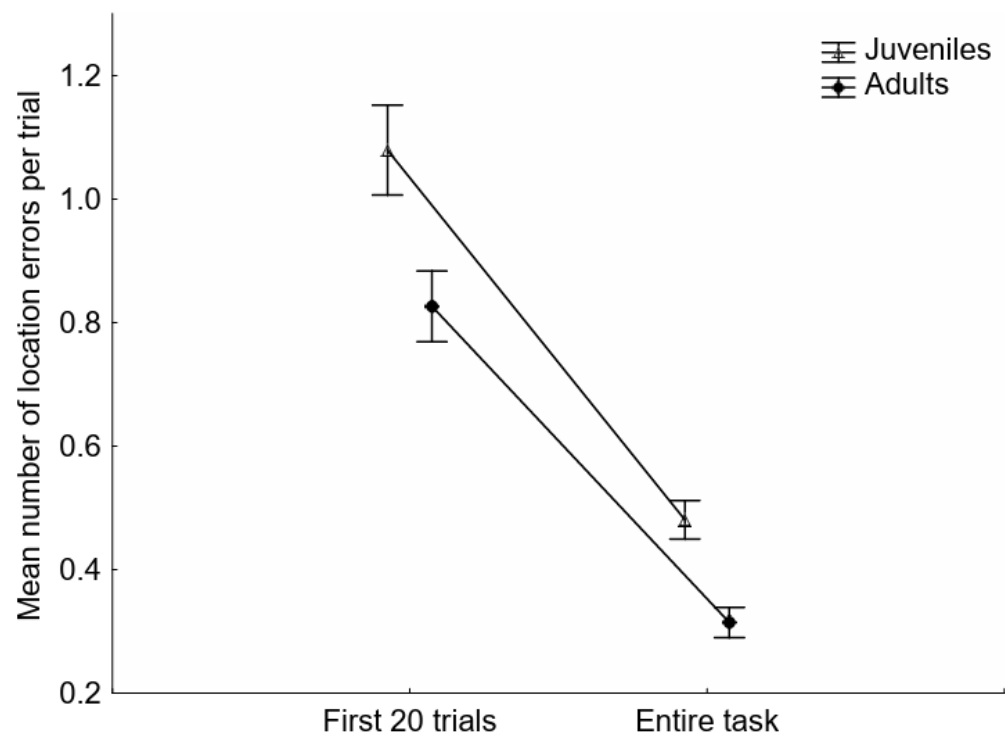


Fig. S2

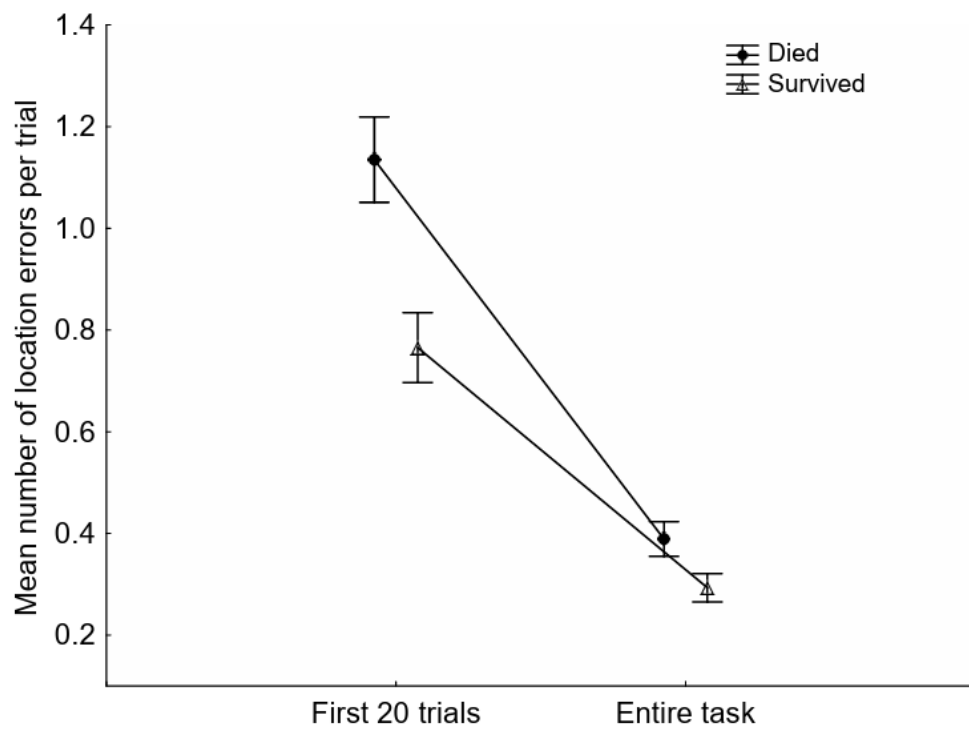


Table S1

	Initial learning	Reversal learning
First year birds	28	24
Adults	45	41

Table S1. Sample sizes for cognitive tasks carried out in 2017-18 season.

Chapter 2

Nest construction, ambient temperature and reproductive success in a cavity-nesting bird

Benjamin R. Sonnenberg^{1*}, Carrie L. Branch^{1,2}, Angela M. Pitera¹, Lauren M. Benedict¹, Vladimir V. Pravosudov¹

¹University of Nevada Reno, Department of Biology and Ecology, Evolution, and Conservation Biology Graduate Program, Reno, NV, USA

²Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA

*Corresponding author

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Abstract

Nest construction is a widespread behaviour that is a critical fitness investment in many taxa. Nests provide stable environments for offspring production, development and at times adult survival. Avian nest structure and composition has been linked to insulative properties and the reduction of energetic requirements for incubating females. However, it is unclear how and why nest structure varies across landscapes within and between species, especially in cavity-nesting species. Here we examined nest size in a secondary cavity nester, the mountain chickadee, *Poecile gambeli*. Nests were measured across two years at two elevations varying in ambient temperature to test whether variation in nest size was associated with local ambient temperatures and internal nest cavity microclimate. Despite both elevations having high variation in ambient temperature during nest building in both years, there were no significant relationships between multiple nest measurements and temperature. Nest size was highly consistent between years within females, suggesting that individual females are the best predictor of nest size. Large variation in nest size, internal nest cavity microclimate temperature and ambient temperature during development in one year was not associated with significant differences in reproduction; but fluctuating asymmetry, a measure of developmental stress, was significantly lower in fledglings at the higher-elevation site independent of temperature. However, there were no significant differences between elevations in the second year for any reproductive parameter. Overall, our results suggest that chickadees do not adjust nest size in response to large differences in

ambient or microclimate temperature and that large variation in nest size, both across and within sites, is a result of consistent individual preferences, which do not seem to have clear fitness consequences.

Introduction

Nests are constructed by animals across invertebrate and vertebrate taxa to hold eggs, young, resources or adults (Deeming & Reynolds 2015, Downing & Jeanne 1986, Hansell 1984, Noirot & Darlington 2000, Rushbrook et al. 2008). Nest use can be as varied as colonial insects using their nest structures throughout the entire life of a colony to many fish species constructing temporary nests for both courtship and raising progeny (Hansell 1984, Rushbrook et al. 2008); although the utility differs, the nests for each are critical to survival and reproductive success (Downing & Jeanne 1986, Noirot & Darlington 2000). For many species, nests and their construction are required for successful reproduction, and individuals are expected to adjust their nest structure to maximize their fitness under prevailing environmental conditions. For example, animals may be able to compensate for the effects of colder temperatures by selecting nest sites located in thermal refuges (i.e. areas with higher sun exposure or lower wind exposure), using selective materials with high insulative capabilities, or building larger nests with better overall thermal insulation (Alabrudzinska et al. 2003, Burton 2007, Britt & Deeming, 2011, Crossman, Rohwer, & Martin 2011, Deeming et al. 2012, Mainwaring et al. 2012, Alvarez et al. 2013). Some vertebrates even show conserved and heritable variation in nest construction (Dawson et al. 1988 Lee

1973), although for many taxa it is not clear whether such variation has important fitness consequences. Avian nest structure in particular shows large variation across taxa, from large communal structures to simple scrapes in the ground (Hansell 1984). In many cases, avian nest structure provides a consummate developmental environment, defined by its ability to maintain or assist in maintaining the optimal microclimate for young, both pre- and posthatch (Amininasab et al. 2016, Dawson et al. 2005, Mueller et al. 2019). This microclimate is produced directly by the energetic output of the parent as well as by the thermoregulatory properties of the nest and its location within a habitat (Li & Martin 1991, McGowan et al. 2004, Heenan 2013, Maziarz & Wesolowski 2013). Warmer microclimates may influence parental behaviour by allowing females to have longer off-nest foraging bouts or reduce their own energetic investment (Martin & Ghalambor 1999). While there are numerous differences in nest structure across and within species, the sources of this variation remain elusive (McGowan et al. 2004, Crossman et al. 2011). In birds, there has been support for interspecific variation corresponding to environmental heterogeneity, with species in colder environments with lower ambient temperatures building more insulated nests than those in warmer climates (Britt & Deeming 2011, Crossman et al. 2011, Deeming et al. 2012, Mainwaring et al. 2012). The same pattern associated with differences in ambient temperature appears within species, with individuals at higher latitudes building nests with thicker walls or using more insulative materials, such as grasses, feathers or mammal hair (Alabrudzinska et al. 2003, McGowan et al. 2004, Crossman et al. 2011,

Mainwaring et al. 2012, 2014). While this pattern has been shown in both open cup and cavity-nesting species, less attention has been paid to the latter (Wiebe 2001). There is evidence that cavity-nesting species alter their nesting environment in several ways, including cavity selection and nest size adjustment within the cavity, both of which may have fitness consequences for the incubating females and developing offspring as both choices impact the potential microclimate of the nest (Wachob 1996). This may be due to ambient temperature variation, which was shown to impact at least some cavity nesters; for example, blue tits, *Cyanistes caeruleus*, use different nest cup lining materials throughout the season based upon ambient temperature during nest construction (Mainwaring & Hartley 2008). Moreover, blue tits, but not their close relative great tits, *Parus major*, build larger nests in colder climates (Alabrudzinska et al. 2003, Britt & Deeming 2011). Explanations for this variation in nest construction and composition in these closely related cavity-nesting species have been quite diverse, with fitness consequences linked to more than variation in ambient temperature. For instance, the mass of great tit nests is dependent upon their distance from the nest cavity hole, as this distance reduces detection by predators and thus predation events (Mazgajski & Rykowska 2008). In addition, great tit nests with a higher percentage of moss had larger clutches, and nests with more feathers and sticks had nestlings in poorer condition (Alvarez et al. 2013). So, it is evident that nest size and composition in cavity nesters can have direct effects on reproductive success in multiple ways. These results suggest that it is likely that there are numerous selective pressures shaping nest structure

across and within avian taxa. Despite considerable attention, available data are equivocal on whether individual variation in nest construction behaviour and size is due to differences in current environments, such as ambient and habitat microclimate temperature, and whether such variation may also be associated with differential reproductive success. Variation in ambient temperature may have significant consequences for nestling development, and adjusting nest size to compensate for such variation may be critical to avoid potential negative effects. Nevertheless, it is unclear whether changes in ambient temperature and what range of such changes may be detrimental. It has been hypothesized that warming temperatures, due to overall climate change, may have dire consequences for avian populations in this regard, yet data are lacking (Mueller et al. 2019). Montane environments present a convenient system to investigate potential relationships among ambient temperature, nest structure and reproductive success within species. Ambient temperature changes rapidly and predictably along an elevational gradient, with higher elevations generally characterized by lower temperatures during the laying period (Kozlovsky et al. 2018). Here, we used obligate secondary cavity nesters, mountain chickadees, *Poecile gambeli*, to investigate whether between- and within-elevation differences in ambient temperature and nestbox microclimate (defined as temperature within a given nestbox) are associated with variation in nest size, and whether this variation is associated with differences in reproductive success. Mountain chickadees are highly resident along montane elevational gradients, and breeding individuals are exposed to a wide array of climatic conditions during

spring and early summer months (Kozlovsky et al. 2018). For instance, at high elevations, chickadees commonly begin nest construction when there is still extensive snow cover on the ground (mean snow depth: 2018: 170 cm 2019: 323 cm), while snow only persists at low elevations during years with above-average precipitation (mean snow depth: 2018: 0 cm; 2019: 87 cm; Kozlovsky et al. 2018). In addition, breeding success varies within elevations from year to year depending on climatic conditions, such as mean snow depth in spring and local ambient temperatures (Kozlovsky et al. 2018). Here we specifically tested the hypotheses that (1) chickadees build deeper nests at high elevation to compensate for overall lower ambient temperatures and (2) chickadees at both high and low elevations build deeper nests in response to lower local ambient and nestbox microclimate temperatures during early nesting periods.

Methods

Study Site

All fieldwork for this study was conducted in 2018 and 2019 at the Sagehen Experimental Forest, California, U.S.A. At our long-term study sites, at two elevations ('low' ca. 1900 m; 'high' ca. 2400 m), we maintain approximately 350 nestboxes divided between each site (Kozlovsky et al. 2018). We monitor nestboxes starting in late April or early May, depending on the snowpack, for nest building, egg laying, incubation initiation and hatch day. Nests are visited at a minimum on a weekly basis throughout the entire reproductive period. To assess nestling condition, we recorded body mass and fluctuating asymmetry in all

nestlings at day 16 posthatch. Day 16 posthatch is used in order to prevent force fledging of the young as they naturally fledge during day 20-22 posthatch. Fluctuating asymmetry is a proxy of developmental stress, which we estimated by comparing the length of the outer rectrices. Birds with more asymmetry (measured in mm) are considered to have experienced higher levels of developmental stress (Palmer & Strobeck 1986, Eeva et al. 2000, Sillanpaa et al. 2010). Adult birds are banded during the spring and autumn months at the site with colour plastic leg bands and a single passive integrated transponder (PIT) tag in order to track individuals across years for both reproductive success and other behavioural work at the site. Nestlings were banded with a single metal numeric band issued by the U.S. Geological Survey (USGS) Bird Banding Laboratory (BBL).

Temperature Data

Ambient temperatures and snow depths for the 2018 and 2019 breeding seasons (May-July) were taken from highly accurate United States Department of Agriculture Natural Resources and Conservation Service SNOTEL stations located within or in very close proximity to our study sites: 541-Independence Lake (directly in the middle of our high-elevation site), 540-Independence Camp and 539-Independence Creek (two stations near our low-elevation site). SNOTEL stations record hourly temperature (C) and snow depth (cm) measurements throughout the year. We estimated mean ambient temperatures during nest building (when nest building was first detected in the nestbox to the onset of incubation), incubation (12-day period) and posthatch (16-day period) using a

custom Perl program (by B.R.S.). We estimated mean microclimate temperature inside the nestbox for the incubation and posthatch periods using Thermochron IButtons glued to the upper right inside corner of each nestbox. Microclimate data was only collected in the 2018 season. These data loggers recorded temperature (C) and were deployed on day 1 of incubation and collected on day 16 posthatch. Since chickadees sometimes start building a nest and then abandon the nest before its completion, we only installed data loggers when birds initiated incubation and not when we detected the first sign of nest building. Mean microclimate temperatures during incubation and the posthatch periods were estimated using a custom Perl program (by B.R.S.). Daily snow depth was collected for both 2018 and 2019 for the overall nest building periods at both elevation sites.

Nest Measurements

We measured nest height at each of the four corners of the nestbox using a ruler through a door at the top of the box and averaged these estimates to obtain a uniform measure of nest depth (Fig.1a). Nestbox sizes are consistent across both sites (Fig.1). Nests were measured as soon as the female was detected actively incubating as this is when females first form the nest cup. We used small wooden dowels (2 mm diameter) to measure nest cup depth. Each dowel was inserted into the nest cup between the eggs and a small wooden ruler placed over the top of the nest cup. We placed a mark on the location where each dowel met the ruler at a perpendicular angle, denoting the exact depth of the nest cup (Fig. 1b). The dowel was then carefully removed from the nestbox

and measured from the mark. We measured nests from both the 2018 and 2019 breeding seasons to evaluate consistency of nest size in the same individuals. Females are the sole nest builders and incubators in this species (McCallum et al. 1999), and sexually mature individuals that had been detected and successfully bred in a nestbox in at least one previous season prior to the study were defined as experienced, while first-time breeders were defined as inexperienced (Branch et al. 2019).

Statistical analysis

All data was analysed using linear models in R statistical software (R v.3.5.2, R Foundation for Statistical Computing, Vienna, Austria).

Temperature comparisons across and within elevations

For comparisons between ambient temperature and microclimate (temperature inside the nestbox) within the same elevation during the same developmental periods, we fitted linear mixed effects models using the R packages 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017). We used mean temperature over the developmental period (estimated using hourly temperatures) as the dependent variable, elevation as an independent variable and nestbox as a random variable. For comparisons between the ambient and microclimate temperatures between elevations across developmental periods, we used mean temperatures from each box as a dependent variable and elevation as an independent variable in an ANOVA analysis using the R package 'car' (Fox & Weisberg, 2019).

Temperature as a predictor of either nest size or nestling characteristics

To determine relationships between nest size, ambient temperature and microclimate, nest characteristics (e.g. nest depth and cup depth) were used as dependent variables with elevation and mean temperature over each developmental period (nest building, incubation and posthatch) as independent variables. ANOVAs were used to compare nest size between elevations. Relationships between temperature, elevation, nest size and reproductive parameters were evaluated using linear models in the base R package. Elevation, temperature and nest size were used as independent variables, and clutch size, brood size, mean nestling mass and mean fluctuating asymmetry were the dependent variables. ANOVAs were used to compare nestling characteristics between and across elevations.

Nest structure across years

Consistency in nest size within females was estimated first by using a simple linear regression to compare the nest depth and nest cup depth between the 2 years of study controlling for box reuse. Additionally, we used the R package 'rptR' (Stoffel et al. 2017) to calculate nest size repeatability across years within the same females (Lessells & Boag 1987). We calculated an R (repeatability) score using a Gaussian distribution and parametric bootstrapping (nboot = 1500) in order to obtain 95% confidence intervals for the estimate. Individual repeatability estimates were calculated for both nest depth and nest cup depth.

Ethical Note

This research has followed the guidelines of the Institutional Animal Care and Use Committee of the University of Nevada, Reno (Protocol No. 00603), as well as local and federal guidelines (California Department of Fish and Wildlife Permit D-0011776516-4). As this was a field study, adult animals were banded and immediately released. We did not detect any detrimental effects of nestbox monitoring or tagging and only did so when environmental conditions were favourable. Juvenile animals were handled as quickly as possible and were only processed when conditions of the nestling and the environment were satisfactory.

Results

Ambient Temperatures

Mean ambient temperatures during the 2018 season varied predictably across the entire reproductive cycle, with the warmest temperatures arriving later in the season (Fig. 2a). The mean ambient temperature during the nest-building period did not differ significantly between elevations (high elevation: N = 38, mean = 7.62 °C; low elevation: N = 58, mean = 7.98 C; $F_{1,94} = 3.35$, $P = 0.07$, $R^2_{adj} = 0.02$). Mean ambient temperature during incubation, however, was significantly lower at low elevation (N = 27, mean = 10.52 °C) compared to high elevation (N = 33, mean = 10.82 °C; $F_{1,58} = 4.047$, $P = 0.048$, $R^2_{adj} = 0.049$). Similarly, mean ambient temperature at the 16-day posthatch period was also significantly lower at low elevation ($F_{1,55} = 68.501$, $P < 0.001$, $R^2_{adj} = 0.547$; higher elevations: N = 32, mean = 14.32 °C; low elevation: N = 25, mean = 12.27 °C; Fig. 2a).

Mean ambient temperatures in the 2019 season also varied predictably across the reproductive cycle, with the warmest temperatures arriving later in the season as expected, but with several elevation-related trends reversing, likely due to the birds at high elevation having a much later breeding time onset in 2019 (27 May) compared to 2018 (14 May) (Fig. 2b). The mean ambient temperature for the nest-building period was significantly lower at low elevation (N = 65, mean = 5.64 °C) compared to that at high elevation (N = 30, mean = 10.07 °C; $F_{1,93} = 224.96$, $P < 0.001$, $R^2_{adj} = 0.16$).

Mean daily maximum and mean daily minimum ambient temperatures (representing the extreme temperatures experienced over each period) are reported in the Supplemental materials.

Microclimate Temperatures in 2018

Mean microclimate temperature during incubation was significantly higher at low elevation (N = 27, mean = 12.60 °C; $F_{1,58} = 19.56$, $P < 0.001$, $R^2_{adj} = 0.239$) than at high elevation (N = 33, mean = 11.39 °C), but did not differ significantly between elevations during the posthatch period (low elevation: N = 23, mean = 15.25 °C; high elevation: N = 32, mean = 15.34 °C; $F_{1,53} = 0.092$, $P = 0.76$, $R^2_{adj} = -0.017$; Fig. 3). The mean microclimate temperatures were significantly higher than the mean ambient temperatures during both incubation ($F_{1,59} = 75.02$, $P < 0.001$, $R^2_{adj} = 0.308$) and the posthatch periods ($F_{1,54} = 177.42$, $P < 0.001$, $R^2_{adj} = 0.41$).

The mean microclimate temperatures for each box were highly and positively associated with the mean ambient temperatures for each box for both

the incubation ($\beta = 0.75$, $t = 3.01$, error = 0.24, $F_{1,58} = 9.19$, $P < 0.01$, $R^2_{\text{adj}} = 0.12$) and the posthatch periods ($\beta = 0.48$, $t = 4.19$, error = 0.09, $F_{1,53} = 24.15$, $P < 0.001$, $R^2_{\text{adj}} = 0.30$), indicating that ambient temperature is a reliable proxy for microclimate.

Mean daily maximum and mean daily minimum microclimate temperatures (representing the extreme temperatures experienced over each period) are reported in the Supplemental materials.

Snow Depth

Snow depths over the duration of the entire nest-building period were significantly lower at both elevations in 2018 compared to 2019, with mean snow depth over 100cm persisting into the incubation period at high elevation in the 2019 season ($F_{2,157} = 621.7$, $P < 0.001$; year: $F_{2,157} = 80.79$, $P < 0.001$, $R^2_{\text{adj}} = 0.81$, Fig. 4).

Nest Size

The average nest depth in 2018 ranged from 3.38 cm to 11 cm, with a mean of 6.45 ± 0.18 cm (Fig. 5a). Nest depth did not differ significantly between low and high elevations ($F_{1,83} = 0.888$, $P = 0.349$, $R^2_{\text{adj}} = -0.001$). Additionally, there were no significant differences in nest depth between experienced females ($N = 52$) and females detected breeding for the first time (inexperienced females; $N = 41$; $F_{2,55} = 0.845$, $R^2_{\text{adj}} = 0.031$, $P = 0.362$) when controlling for elevation ($F_{2,55} = 2.971$, $R^2_{\text{adj}} = 0.031$, $P = 0.09$).

The average nest depth in 2019 ranged from 3.35 cm to 10.50 cm, with a mean of 6.19 ± 0.15 cm across both elevations (Fig. 5b), with significantly

smaller nests at high elevations (low elevation: $N = 65$, mean = 6.62 cm; high elevation: $N = 30$, mean = 5.28cm; $F_{2,92} = 17.7$, $P < 0.001$). Previous breeding experience was again not significantly associated with nest size (experienced females: $N = 70$; inexperienced females: $N = 23$; $F_{2,92} = 0.52$, $P = 0.47$, $R^2_{adj} = 0.16$). Nest depth did not differ significantly across years while controlling for elevation (nest depth: $F_{2,188} = 2.14$, $P = 0.14$; elevation: $F_{2,188} = 2.17$, $P = 0.14$, $R^2_{adj} = 0.008$).

Nest cup depth in 2018 ranged from 3 cm to 5.5 cm, with a mean of 4.38 ± 0.06 cm (Fig. 5c). There were no significant differences between low and high elevations or with the experience of the female (elevation: $F_{2,55} = 2.20$, $P = 0.14$; female experience: $F_{2,55} = 0.195$, $P = 0.65$, $R^2_{adj} = 0.007$). Nest cup depth in 2019 ranged from 2.9 cm to 5.4 cm, with a mean of 4.28 ± 0.05 cm (Fig. 5d), and nests at low elevations had significantly deeper nest cups compared to those at high elevations ($F_{2,92} = 25.63$, $P < 0.001$). There were no significant differences in cup depths between experienced and inexperienced females ($F_{2,92} = 1.54$, $P = 0.21$, $R^2_{adj} = 0.21$). Nest cup depth did not differ across years while controlling for elevation (nest cup depth: $F_{2,188} = 1.47$, $P = 0.23$; elevation: $F_{2,188} = 2.02$, $P = 0.16$, $R^2_{adj} = 0.004$).

Nest depth was not significantly associated with the average nest building time while controlling for female experience in the 2018 (average build time: high elevation = 18 days, low elevation = 23 days; elevation: $F_{3,89} = 0.73$, $P = 0.39$; female experience: $F_{3,89} = 2.48$, $P = 0.12$; build time: $F_{3,89} = 0.09$, $P = 0.76$, $R^2_{adj} = 0.006$) or in 2019 (high elevation = 14 days, low elevation = 22 days; elevation:

$F_{3,89} = 15.12$, $P < 0.001$; female experience: $F_{3,89} = 0.39$, $P = 0.53$; build time: $F_{3,89} = 0.52$, $P = 0.47$, $R^2_{\text{adj}} = 0.16$).

Microclimate, Ambient Temperature and Nest Size

Nest depth and nest cup depth were not significantly associated with mean microclimate temperature during the incubation or the posthatch period (Table 1, Fig. 6). In addition, mean daily maximum and mean daily minimum microclimate temperatures (representing experienced temperature extremes) were also not significantly associated with either nest depth or nest cup depth over these same periods (see Supplementary materials).

Nest depth and nest cup depth in both 2018 and 2019 were also not significantly associated with mean ambient temperature during the nest-building, incubation or posthatch period (Table 1, Fig. 7). The mean daily maximum and minimum temperatures were also not significantly associated with either nest depth or nest cup depth in either year or over any development period measured (see Supplementary materials).

Repeatability of Nest Size in Females

54 females re-nested between the 2 years of the study, 69% of which nested in a different nestbox between years while the remaining 31% nested in the same box both years. Accounting for box reuse, individual females were strong predictors of nest size from year to year ($\beta = 0.672$, $t = 5.497$, error = 0.122, $F_{2,51} = 5.497$, $R^2_{\text{adj}} = 0.351$, $P < 0.001$; Fig 8a), which remained true whether females stayed in the same nestbox or moved to a new box between years of the study ($F_{2,51} = 0.817$, $p = 0.37$). Nest cup depth was also highly

repeatable across seasons, although the effect was mainly driven by low elevation ($\beta = 0.25$ (partial coefficients: high elevation: $\beta = 0.17$, low elevation $\beta = 0.32$), $t = 5.80$, error = 0.54, $F_{2,51} = 4.96$, $R^2_{adj} = 0.1$, $P = 0.03$; Fig. 8b), and remained true whether females stayed in the same nest box or moved to a new box ($F_{2,51} = 2.68$, $P = 0.17$).

Repeatability estimates also showed significant individual repeatability of nest depth (repeatability estimate: 0.58; CI: 0.36, 0.73; $P < 0.05$) in individual females between the 2 years of study.

Reproduction, Nest Size, and Temperature

Variation in nest depth and microclimate was not significantly associated with differences in clutch size, brood size, mean nestling mass, or mean fluctuating asymmetry (Table 2, Fig. 9a-d). The means of both daily maximum and daily minimum microclimate temperatures were also not significantly associated with any reproductive parameters (see Supplementary materials).

Variation in nest depth and mean ambient temperature across both 2018 and 2019 were also not significantly associated with clutch size, brood size, mean nestling mass or mean fluctuating asymmetry (Table 2). The mean daily maximum and minimum ambient temperatures were similarly not significantly associated with any reproductive parameters (see Supplementary materials).

Mean fluctuating asymmetry at day16 was significantly lower at higher elevations in 2018 ($F_{3,51} = 7.42$, $P < 0.01$, $R^2_{adj} = 0.07$; Fig 10a) but not in 2019 ($F_{1,93} = 0.49$, $P = 0.48$, $R^2_{adj} = -0.005$; Fig. 10b).

Discussion

Nest size exhibited large individual variation both between and across elevations, but such variation was not explained by similarly large differences in mean nestbox microclimate temperature in a single season or in mean ambient temperatures across multiple seasons. This remained true for the temperature extremes for both microclimate and ambient temperatures across years. Nest size was not significantly different between elevations in 2018, but nests were significantly smaller at high elevation in 2019, likely due to the persistence of high snow cover, which resulted in lower substrate availability. The only significant predictor of nest size was individual females, which constructed highly repeatable nests between years. At the same time, none of the reproductive parameters were significantly associated with variation in nest size, nestbox microclimate temperature or ambient temperature in either year despite large variation in both.

Altogether, our results suggest that large variation in nest size in mountain chickadees is likely due to either genetic differences among females (Lee 1973, Dawson et al. 1988) or, potentially, some unique female experiences, such as females imprinting on their own natal environments. Natal imprinting on nest structure has been documented, for example, in species of brood parasites as mechanisms of host nest identification (Teuschl et al. 1998). Large variation in nest size may be maintained if it has no significant fitness consequences for the developing offspring or the parents, which appears to be the case with chickadees, considering that large variation in microclimate nestbox temperatures (9.73 - 17.54 °C over the entire nesting period) and in ambient

temperatures (2018: 5.55 -15.08 C; 2019: 3.94 - 15.35 °C) was not significantly associated with any reproductive parameters.

One of the striking results of our study was that differences in nest size were unrelated to large variation in microclimate temperature or ambient temperature, which did not support our major predictions. Previous results showed that species closely related to mountain chickadees may construct different nests in response to temperature variation (both microclimate and ambient) across latitudinal gradients (Mainwaring & Hartley 2008, Britt & Deeming 2011, Alvarez et al. 2013). These reported differences ranged from the overall mass of the nest and its composition to differences in the mass and insulative properties of the nest cup lining (Mainwaring & Hartley 2008, Britt & Deeming 2011, Alvarez et al. 2013). Despite the differences in yearly temperatures during the onset of the breeding season for the chickadees in the Sierra Nevada, there were no significant differences in the overall nest size.

We, unfortunately, were unable to measure differences in nest mass or nest cup lining due to the damage caused by maturing nestlings before fledging, and it was impossible to remove the nest from the nestbox prior to incubation without potential damage to the nest. Indeed, it may be nest composition instead of size that is important, and this may explain the lack of any relationship between overall size and local temperatures in this study. Smaller nests, if made from superior materials, could be favoured in colder temperatures. We do not think this is the case, however, as mountain chickadee nests are of simple construction and many of the small nests were made almost entirely of small

sticks and wood pine chips, which have little insulative capabilities. In addition, high repeatability of nest size within individual females across years and in different thermal environments are not consistent with this explanation.

Our results do not support the hypothesis that chickadees use either ambient or microclimate temperature as a cue to adjust nest size, but instead suggest that these birds may be resilient to relatively large variation in temperature and that nest size may potentially have no direct functional significance. Female chickadees are the sole nest builders and incubators and may be able to compensate for differences in temperature with behaviours such as increasing the length of their incubation bouts (McCallum et al. 1999).

There was a high degree of variation in snow depth, especially at high elevations, during the onset of breeding from year to year. It appears that the majority of chickadee nest materials are gathered from the ground; thus, limited and varying access to these resources may have led to weak selection on nest construction but perhaps strong selection on female compensatory mechanisms. Nest depth was significantly smaller at high elevation in 2019, when snow levels were higher (snow depth > 100cm) during the nest-building period than in 2018, which is consistent with this conclusion. In addition, male chickadees feed nesting females, and such supplemental feeding may influence the length of incubation bouts. Past studies in closely related species showed that male supplemental feeding led to earlier onset of nest construction and egg laying (Martin & Ghalambor 1999, Smith et al. 2013), and that the health of females was

a predictor of nest mass, with healthier females building nests of higher mass (Tomas et al. 2006). We did not measure these variables in the present study.

Global climate change is characterized in many environments by a steady increase in ambient temperature, which may affect elevational gradients such as the Sierra Nevada, first, by shifting optimal temperature limits for species residing at high elevations (Wilson et al. 2005, Rangwala & Miller 2012, Skagen & Adams 2012). It has been documented that higher temperatures may result in drastic changes in embryonic development and, in extreme cases, even death of the young (Moreng & Shaffner 1951, Berntsen & Bech 2016). However, small artificial increases in temperature (1 - 5 °C) in the nests of multiple cavity-nesting species resulted in both positive and negative fitness effects on the offspring (Dawson et al. 2005, Mueller et al. 2019). Results of our study provided no evidence to suggest that the large ambient temperature variation (2018: nest building: 5.55 - 11.88 °C; incubation: 9.7 - 15.1 °C; posthatch: 13.1 - 17.5 °C; 2019: nest building: 4.75 - 13.71 °C; incubation: 3.94 - 14.38 °C; posthatch: 9.49 - 15.35 °C) across elevations was associated with detectable reproductive differences, despite higher microclimate temperatures during incubation at low elevations in 2018. In addition, we found no evidence to suggest that the size of the nest had any detectable effect on reproductive parameters in either season. However, long-term monitoring of the reproductive effort of mountain-dwelling species may lead to significant developments in the understanding of how resident species may respond to changes in climate.

Fluctuating asymmetry, a measure of developmental stress, can be an indicator of the quality of the developmental environment (Palmer & Strobeck, 1986, Eeva et al. 2000, Sillanpaa et al. 2010). Even though the degree of fluctuating asymmetry was not explained by microclimate temperature during development or by the size of the nest, fledglings at high elevation had lower asymmetry in 2018. Some previous research suggests that females that undergo stress during nest construction may build different nests that lead to less fit young (Tomas et al. 2006). However, this does not seem to be the case in our study. It is likely that other differences in developmental environment, such as parent-supplied prey quality or nest parasite load (both under current investigation in this system), may be possible drivers behind elevation-related differences in fluctuating asymmetry. At the same time, there were no significant differences in fluctuating asymmetry between elevations in 2019 despite large variation in ambient temperature and nest size, adding further support to this claim.

We measured microclimate temperature only in 2018, and we began doing so during the incubation period. The reason we did not deploy iButtons during nest building was because chickadees frequently start building a nest and then abandon it, moving to a different nestbox before egg laying. So, we deployed iButtons only once a female had started incubation, as these temperatures were also predicted to be related to nest size. At the same time, mean ambient temperature was strongly correlated with mean microclimate temperature during incubation and posthatch in 2018, suggesting that ambient temperature is a good

proxy for microclimate temperature. Ambient temperature itself has been shown to be a strong cue, as previous studies report that it can affect both nest structure and size (Wachob 1996, Wiebe 2001, Deeming et al. 2012, Mainwaring et al. 2014), yet our study suggests that variation in both ambient temperature and microclimate temperature is not associated with differences in either nest size or reproduction.

A critical result of our study in the face of no significant associations between temperature and nest size was that nest size was highly repeatable within individual females, with some females consistently building deeper nests while others built smaller nests across years. This result may have several potential explanations, including a genetic basis for nest size, which has been described in other taxa and has long been suspected in many avian taxa (Lee, 1973, Dawson et al. 1988, Walsh et al. 2010). Alternatively, early experiences may influence nest construction decisions in chickadees, produced via imprinting on their own natal environment or on their first construction attempt, leading to consistency in nest size across years. Due to limited information on the pedigrees of chickadees at our site, we are unable to assess effects of heritability or imprinting on chickadee nest construction. However, given large variation in nest size and a lack of associated fitness consequences, it appears that variation in nest size may not be under selection. Most importantly the fact that large variation in nest size across 2 years was not associated with the equally large variation in temperature but was predicted by individual females strongly supports our conclusion that nest size was independent of ambient or microclimate

temperature. In fact, previous studies relating nest size and climate associated with latitude (Crossman et al. 2011, Mainwaring et al. 2014) may have been a result of cultural differences in populations rather than a response to differences in climate, especially if nest construction behaviour has a stronger genetic component than previously believed.

Overall, our results suggest that nest construction is not a significant contributor to variation in reproductive success, and that while the nest itself may be important, nest size may lack functional significance in the mountain chickadee.

Author Contributions

B.R.S and V.V.P conceived of the study and analysed the data. B.R.S, C.L.B., L.M.B., A.M.P. and V.V.P. collected data. All authors co-wrote the manuscript.

Conflict of Interest

We declare no competing interests.

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Tables

Table 1

F statistics for models with nest size parameters as the response variable and elevation and temperature as predictor variables

Model	2018		2019	
	Nest depth	Nest cup depth	Nest depth	Nest cup depth
Elevation	$F_{2,57} = 2.75, P = 0.10$	$F_{2,57} = 1.60, P = 0.21$		
Microclimate during incubation (nestbox temperature)	$F_{2,57} = 1.56, P = 0.22$ $R^2_{adj} = 0.03$	$F_{2,57} = 2.74, P = 0.10$ $R^2_{adj} = 0.04$		
Elevation	$F_{2,52} = 3.50, P = 0.07$	$F_{2,52} = 2.44, P = 0.12$		
Microclimate during posthatch (nestbox temperature)	$F_{2,52} = 0.03, P = 0.86$ $R^2_{adj} = 0.027$	$F_{2,52} = 1.54, P = 0.22$ $R^2_{adj} = 0.04$		
Elevation	$F_{2,93} = 0.63, P = 0.43$	$F_{2,93} = 0.88, P = 0.35$	$F_{2,92} = 15.98, P < 0.01$	$F_{2,92} = 5.69, P < 0.05$
Ambient temperature during nest building	$F_{2,93} = 0.44, P = 0.51$ $R^2_{adj} = -0.007$	$F_{2,93} = 2.25, P = 0.13$ $R^2_{adj} = 0.01$	$F_{2,92} = 0.38, P = 0.54$ $R^2_{adj} = 0.16$	$F_{2,92} = 0.17, P = 0.68$ $R^2_{adj} = 0.20$
Elevation	$F_{2,57} = 2.78, P = 0.12$	$F_{2,57} = 1.59, P = 0.21$	$F_{2,92} = 12.8, P < 0.001$	$F_{2,92} = 28.89, P < 0.001$
Ambient temperature during incubation	$F_{2,57} = 2.37, P = 0.13$ $R^2_{adj} = 0.05$	$F_{2,57} = 2.12, P = 0.15$ $R^2_{adj} = 0.03$	$F_{2,92} = 2.39, P = 0.12$ $R^2_{adj} = 0.18$	$F_{2,92} = 2.85, P = 0.09$ $R^2_{adj} = 0.22$
Elevation	$F_{2,54} = 2.73, P = 0.10$	$F_{2,54} = 1.80, P = 0.19$	$F_{2,92} = 16.89, P < 0.001$	$F_{2,92} = 22.68, P < 0.001$
Ambient temperature during posthatch	$F_{2,54} = 0.12, P = 0.73$ $R^2_{adj} = 0.02$	$F_{2,54} = 0.07, P = 0.79$ $R^2_{adj} = -0.002$	$F_{2,92} = 0.02, P = 0.89$ $R^2_{adj} = 0.16$	$F_{2,92} = 0.15, P = 0.69$ $R^2_{adj} = 0.20$

Significant *P* values are shown in bold.

Table 2

F statistics for models with reproductive parameters as response variables and elevation, temperature (during incubation¹ or posthatch²) and nest size as predictor variables

Model	Clutch size ¹	Brood size ²	Mass ²	Asymmetry ²
2018				
Elevation	$F_{3,56} = 3.79, P = 0.06$	$F_{3,51} = 3.29, P = 0.07$	$F_{3,51} = 0.33, P = 0.59$	$F_{3,51} = 6.53, P < 0.05$
Microclimate (nestbox temperature)	$F_{3,56} = 0.12, P = 0.73$	$F_{3,51} = 0.79, P = 0.37$	$F_{3,51} = 0.02, P = 0.875$	$F_{3,51} = 0.07, P = 0.79$
Nest depth	$F_{3,56} = 0.22, P = 0.64$ $R^2_{adj} = 0.01$	$F_{3,51} = 0.004, P = 0.95$ $R^2_{adj} = 0.01$	$F_{3,51} = 0.91, P = 0.34$ $R^2_{adj} = -0.03$	$F_{3,51} = 0.07, P = 0.78$ $R^2_{adj} = 0.08$
Elevation	$F_{3,56} = 3.64, P = 0.06$	$F_{3,51} = 3.81, P = 0.06$	$F_{3,51} = 0.09, P = 0.77$	$F_{3,51} = 0.80, P = 0.37$
Ambient temperature	$F_{3,56} = 0.00, P = 0.98$	$F_{3,51} = 1.04, P = 0.31$	$F_{3,51} = 0.01, P = 0.91$	$F_{3,51} = 1.68, P = 0.20$
Nest depth	$F_{3,56} = 0.16, P = 0.69$ $R^2_{adj} = 0.01$	$F_{3,51} = 0.01, P = 0.91$ $R^2_{adj} = 0.02$	$F_{3,51} = 0.92, P = 0.34$ $R^2_{adj} = -0.03$	$F_{3,51} = 0.03, P = 0.85$ $R^2_{adj} = 0.11$
2019				
Elevation	$F_{3,91} = 0.56, P = 0.46$	$F_{3,91} = 2.33, P = 0.13$	$F_{3,91} = 0.38, P = 0.54$	$F_{3,91} = 1.04, P = 0.31$
Ambient temperature	$F_{3,91} = 0.48, P = 0.49$	$F_{3,91} = 0.29, P = 0.59$	$F_{3,91} = 0.02, P = 0.89$	$F_{3,91} = 0.02, P = 0.88$
Nest depth	$F_{3,91} = 0.25, P = 0.61$ $R^2_{adj} = -0.002$	$F_{3,91} = 0.06, P = 0.79$ $R^2_{adj} = 0.002$	$F_{3,91} = 0.19, P = 0.66$ $R^2_{adj} = -0.022$	$F_{3,91} = 0.94, P = 0.33$ $R^2_{adj} = -0.02$

Significant *P* values are shown in bold.

Figure Legends

Fig. 1

Nestbox dimensions and guide to nest size measurements: (a) nest depth (cm); (b) nest cup depth (cm).

Fig. 2

Mean ambient temperature (\pm SE) during the nest-building, incubation and posthatch periods in (a) 2018 and (b) 2019.

Fig. 3

Mean 2018 microclimate temperature (\pm SE) during incubation and posthatch periods.

Fig. 4

Snow depth (cm) during each day of the nest-building period for high and low elevations across both 2018 and 2019.

Fig. 5

Mean nest depth (cm) for (a) 2018 and (b) 2019. Mean nest cup depth (cm) for (c) 2018 and (d) 2019.

Fig. 6

Relationships between microclimate temperature and (a, b) nest depth and (c, d) nest cup depth during incubation and posthatch periods, respectively.

Fig. 7

Relationships between ambient temperature and nest depth during (a, b) 2018 and (c, d) 2019 nest-building and incubation periods, respectively.

Fig. 8

Relationship between (a) nest depth and (b) nest cup depth for the same individual females in 2018 and 2019, with size of the point indicating nestbox reuse.

Fig. 9

Relationships between microclimate temperature and (a) clutch size during incubation, (b) brood size during the posthatch period, (c) mean nestling mass during the posthatch period and (d) fluctuating asymmetry during the posthatch period.

Fig. 10

Mean fluctuating asymmetry (\pm SE) between elevations in (a) 2018 and (b) 2019.

Figures

Figure 1

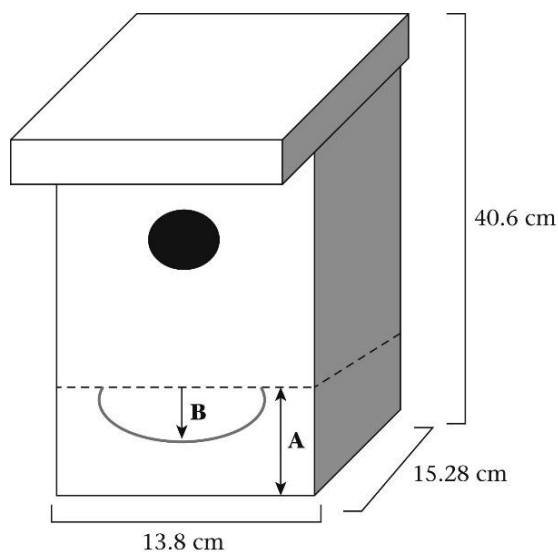


Figure 2

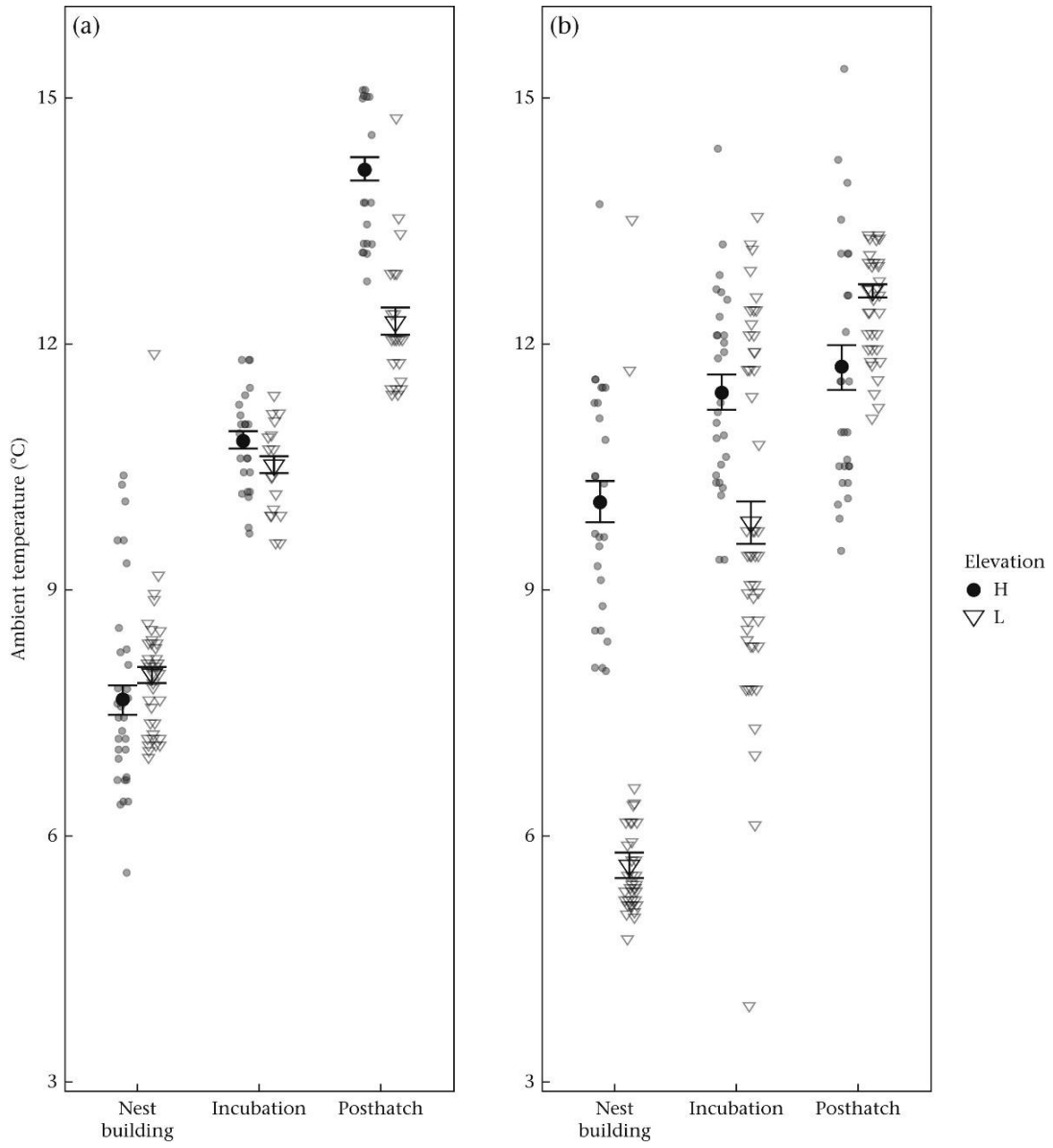


Figure 3

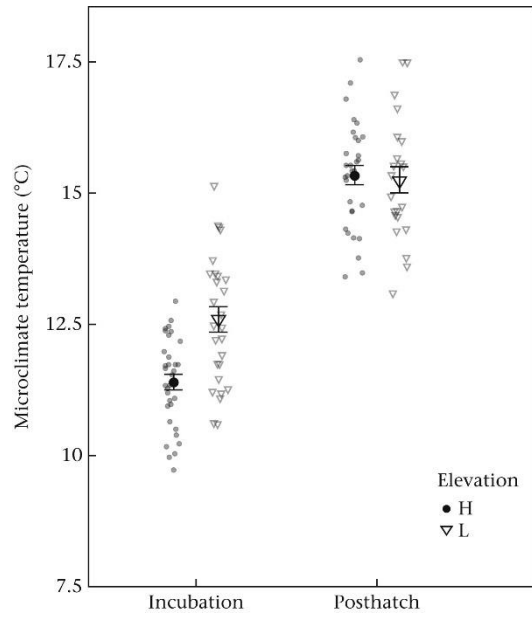


Figure 4

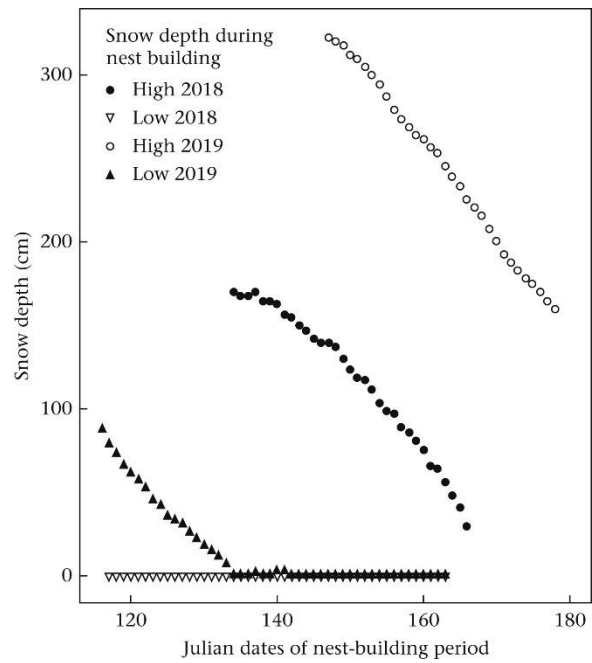


Figure 5

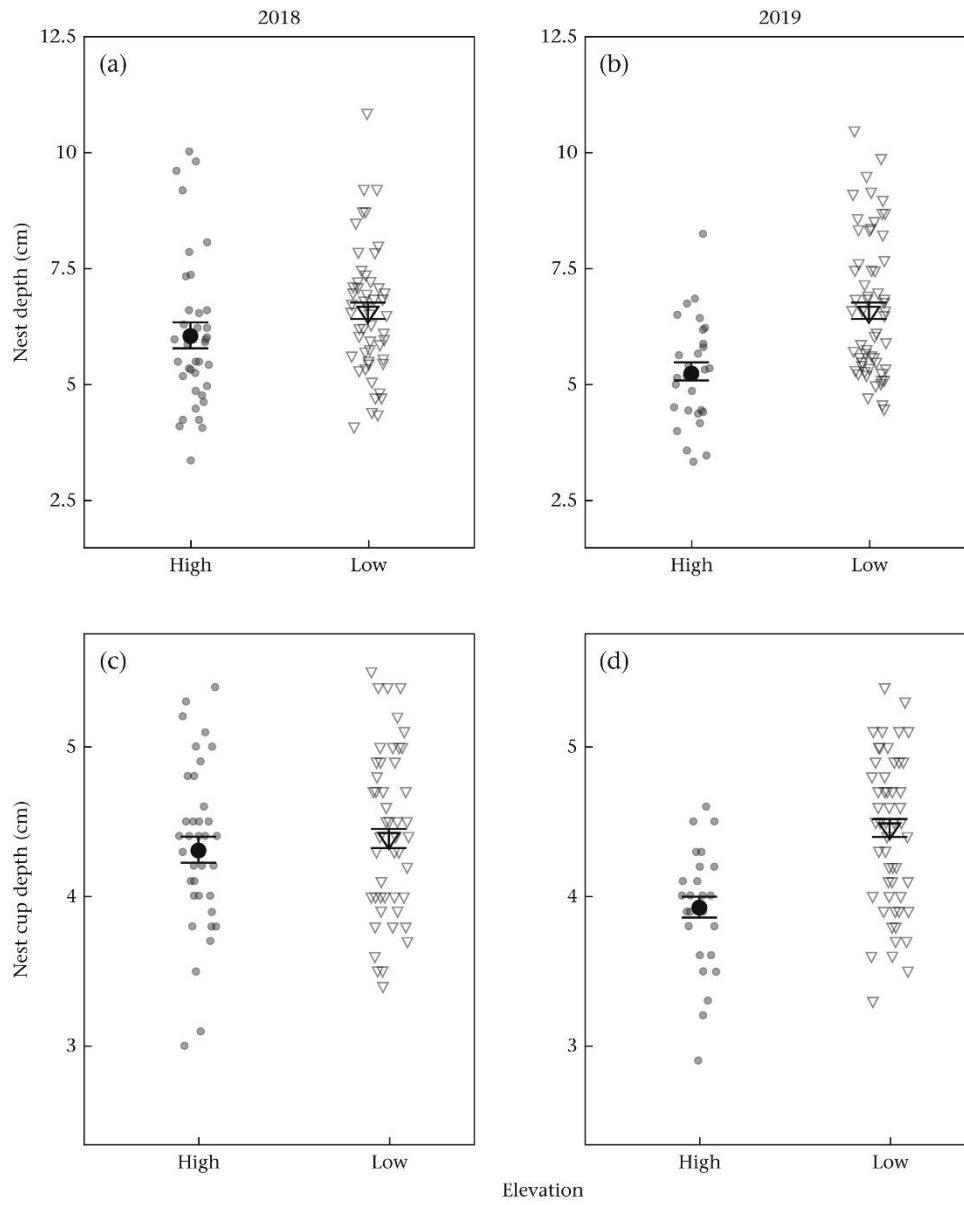


Figure 6

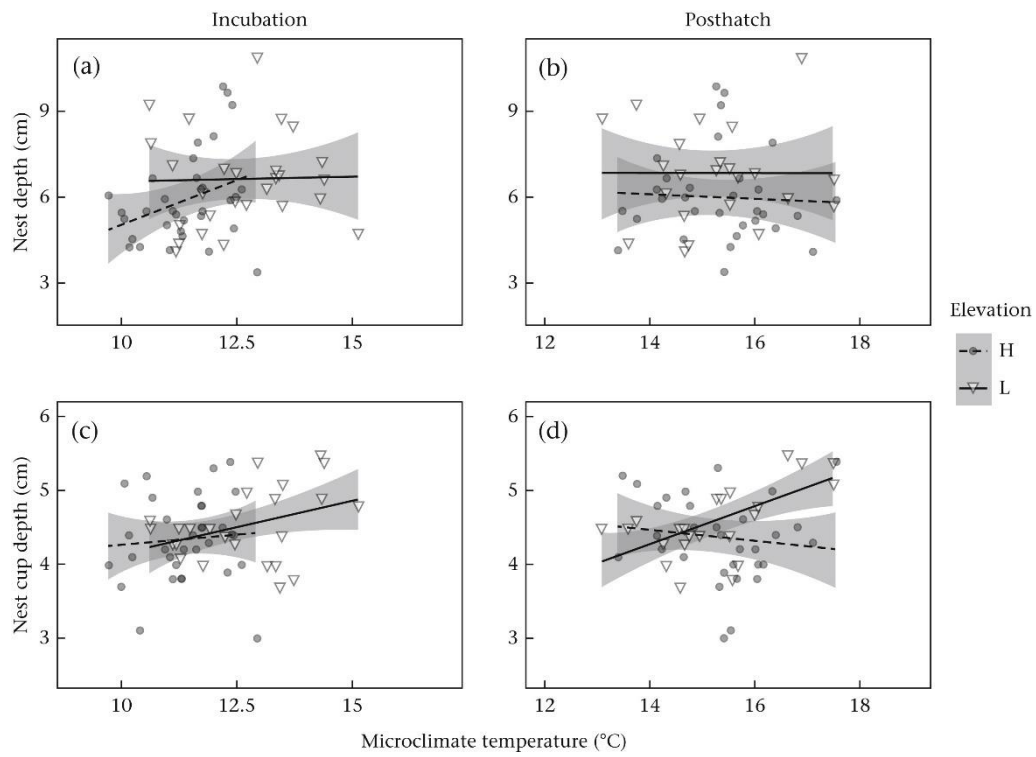


Figure 7

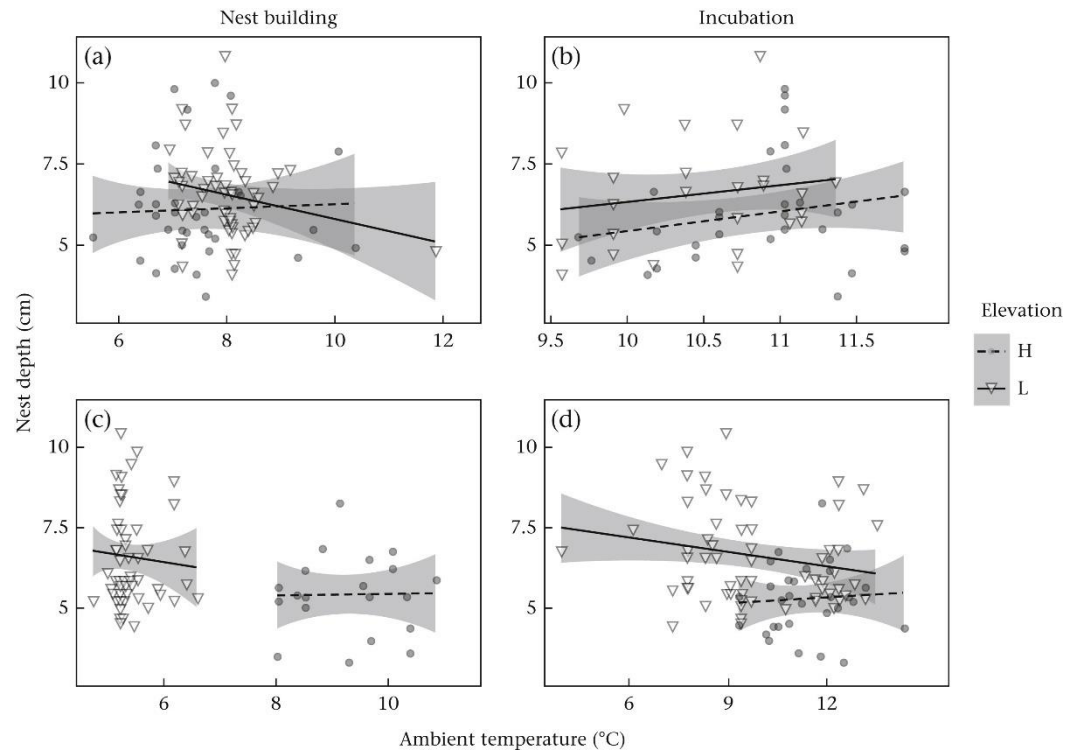


Figure 8

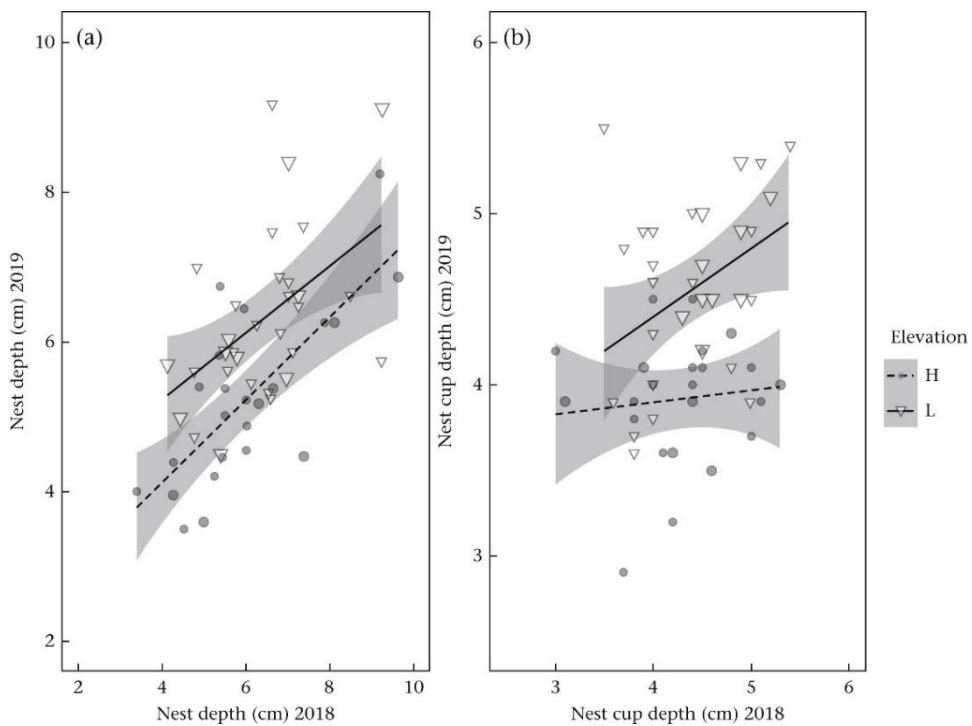


Figure 9

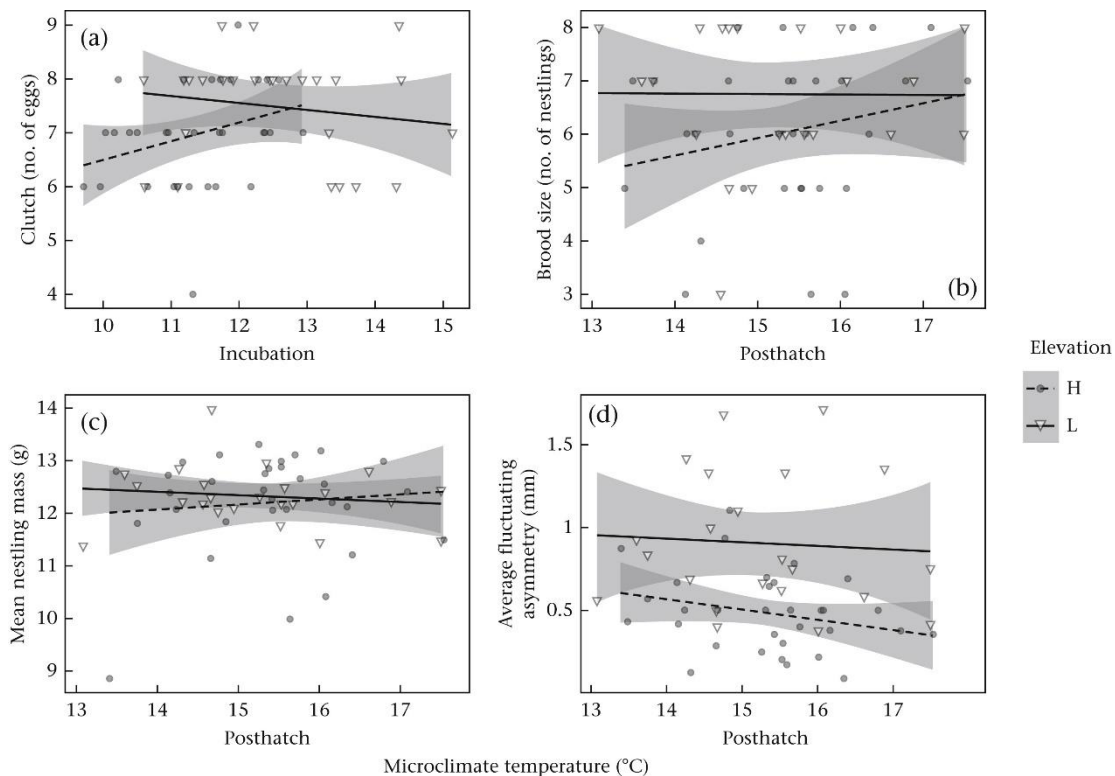
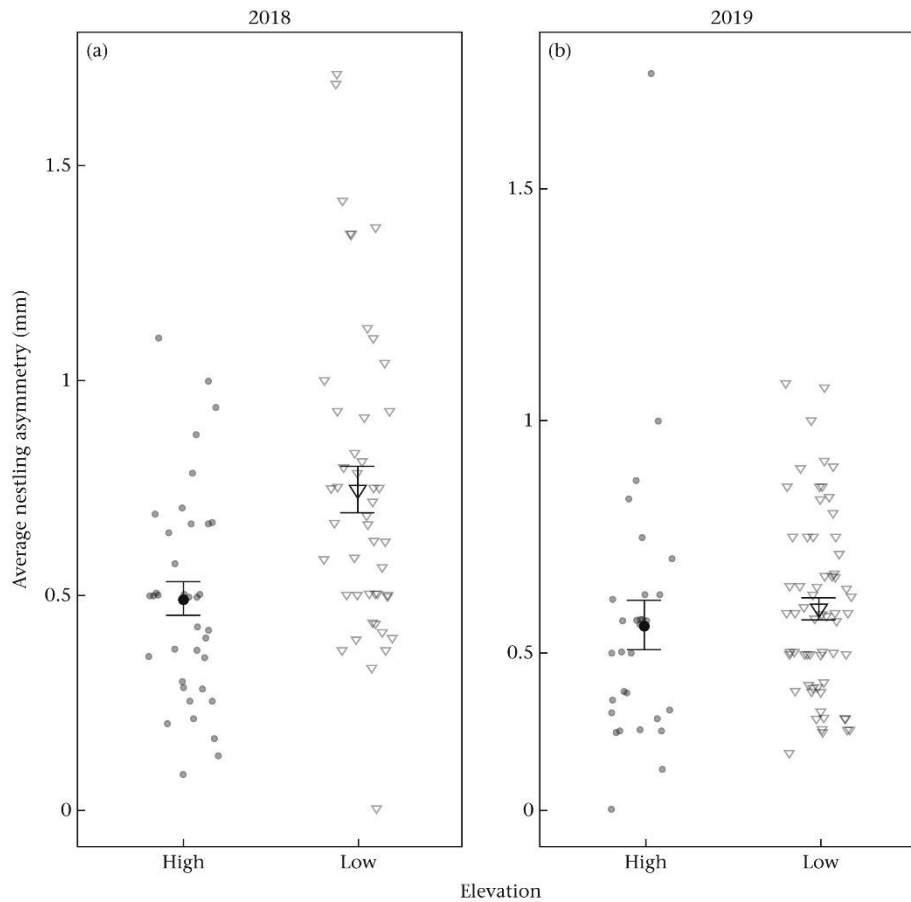


Figure 10

Supplementary materials

Mean Daily Maximum and Minimum Microclimate Temperatures

Mean daily minimum microclimate temperatures during incubation were significantly different between elevations, with low elevation (N = 27, mean = 5.15 °C) being significantly cooler than high elevation (N = 32, mean = 9.22 °C; $F_{1,57} = 191.7$, $P < 0.001$, $R^2_{adj} = 0.77$; Fig. S1a), which is the reverse trend seen when examining the overall average during the period.

Mean daily maximum microclimate temperatures during incubation were not significantly different between elevations (low elevation: N = 27, mean =

22.97 °C; high elevation: N = 32, mean = 23.63 °C; $F_{1,57} = 0.78$, $P = 0.38$, $R^2_{\text{adj}} = 0.004$; Appendix, Fig. S1b).

Mean daily minimum microclimate temperatures during the posthatch period were significantly different between elevations, with low elevation (N = 23, mean = 7.20 °C) being significantly cooler than high elevation (N = 32, mean = 9.23 °C; $F_{1,53} = 35.86$, $P < 0.001$, $R^2_{\text{adj}} = 0.39$; Fig S1a).

Mean daily maximum microclimate temperatures during the posthatch period were significantly different between elevations, with low elevation (N = 23, mean = 26.14 °C) being significantly warmer than high elevation (N = 32, mean = 23.63 °C; $F_{1,53} = 11.0$, $P < 0.01$; Fig S1b).

Mean Daily Maximum and Minimum Ambient Temperatures

Mean daily minimum ambient temperatures were significantly different between elevations during the nest building period, incubation and posthatch periods of 2018 (Fig. S2a) and 2019 (Fig. S2b), with lower elevations consistently experiencing cooler temperatures (Table S1).

Mean daily maximum ambient temperatures in 2018 were significantly warmer at lower elevations during nest building and incubation but not during the posthatch period (Fig. S3a, Table S1).

Mean daily maximum temperatures in 2019 were significantly warmer at higher elevations during nest building and significantly warmer at low elevations during the posthatch period but did not differ significantly between elevations during incubation (Table S1, Fig. S3b).

Mean Daily Maximum and Minimum Microclimate Temperatures and Nest Size

The mean daily maximum temperatures or the mean daily minimum temperatures across all years and developmental periods for both microclimate and ambient temperature were not associated with nest depth or nest cup (Table S2).

Reproduction, Nest Size and Mean Maximum and Minimum Ambient and Microclimate Temperatures

The mean daily maximum temperature or the mean daily minimum temperature across all years and developmental periods for both microclimate and ambient temperature were not associated with clutch size, brood size, mean nestling mass or mean nestling asymmetry (Table S3).

Supplementary Tables

Table S1

F statistics for models with temperature as the response variable and elevation as a predictor

Model	2018		2019	
	Daily max. temperatures	Daily min. temperatures	Daily max. temperatures	Daily min. temperatures
Elevation: Nest building	$F_{1,94} = 24.0, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.20$	$F_{1,94} = 416.1, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.81$	$F_{1,93} = 97.6, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.51$	$F_{1,93} = 636.2, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.87$
Elevation: Incubation	$F_{1,58} = 27.3, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.31$	$F_{1,58} = 915.6, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.94$	$F_{1,93} = 1.09, P = 0.3, R^2_{\text{adj}} = 0.0009$	$F_{1,93} = 418.7, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.82$
Elevation: Posthatch	$F_{1,55} = 0.0002, P = 0.99, R^2_{\text{adj}} = -0.02$	$F_{1,55} = 922.7, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.94$	$F_{1,93} = 169.8, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.64$	$F_{1,93} = 300.7, P < \mathbf{0.001}, R^2_{\text{adj}} = 0.76$

Significant *P* values are shown in bold.

Table S2

F statistics for models with nest size parameters as response variables and elevation, daily maximum and daily minimum temperatures as predictor variables

Model	2018		2019	
	Nest depth	Nest cup depth	Nest depth	Nest cup depth
Elevation	$F_{2,56} = 2.64, P = 0.11$	$F_{2,56} = 1.69, P = 0.19$		
Microclimate daily maximum mean temperature during incubation	$F_{2,56} = 0.63, P = 0.42,$ $R^2_{adj} = 0.017$	$F_{2,56} = 2.35, P = 0.13,$ $R^2_{adj} = 0.03$		
Elevation	$F_{2,56} = 0.05, P = 0.82$	$F_{2,56} = 0.40, P = 0.52$		
Microclimate daily minimum mean temperature during incubation	$F_{2,56} = 0.33, P = 0.56,$ $R^2_{adj} = 0.012$	$F_{2,56} = 0.01, P = 0.90,$ $R^2_{adj} = -0.01$		
Elevation	$F_{2,52} = 2.38, P = 0.12$	$F_{2,52} = 0.64, P = 0.42$		
Microclimate daily maximum mean temperature during posthatch	$F_{2,52} = 0.15, P = 0.69,$ $R^2_{adj} = 0.03$	$F_{2,52} = 2.27, P = 0.13,$ $R^2_{adj} = 0.048$		
Elevation	$F_{2,52} = 1.15, P = 0.28$	$F_{2,52} = 2.93, P = 0.09$		
Microclimate daily minimum mean temperature during posthatch	$F_{2,52} = 0.34, P = 0.56,$ $R^2_{adj} = 0.033$	$F_{2,52} = 0.66, P = 0.41,$ $R^2_{adj} = 0.02$		
Elevation	$F_{2,93} = 0.35, P = 0.55$	$F_{2,93} = 1.60, P = 0.21$	$F_{2,92} = 15.8, P < 0.001$	$F_{2,92} = 9.01, P < 0.001$
Ambient daily maximum mean temperature during nest building	$F_{2,93} = 0.27, P = 0.60,$ $R^2_{adj} = -0.01$	$F_{2,93} = 2.20, P = 0.14,$ $R^2_{adj} = 0.007$	$F_{2,92} = 1.41, P = 0.25,$ $R^2_{adj} = 0.17$	$F_{2,92} = 0.55, P = 0.46,$ $R^2_{adj} = 0.20$
Elevation	$F_{2,93} = 1.43, P = 0.23$	$F_{2,93} = 0.69, P = 0.41$	$F_{2,92} = 1.18, P = 0.28$	$F_{2,92} = 4.60, P < 0.05$
Ambient daily minimum mean temperature during nest building	$F_{2,93} = 0.77, P = 0.38,$ $R^2_{adj} = -0.004$	$F_{2,93} = 1.49, P = 0.22,$ $R^2_{adj} = -0.0006$	$F_{2,92} = 0.29, P = 0.59,$ $R^2_{adj} = 0.16$	$F_{2,92} = 0.14, P = 0.71,$ $R^2_{adj} = 0.2$
Elevation	$F_{2,57} = 0.35, P = 0.56$	$F_{2,57} = 0.03, P = 0.85$	$F_{2,92} = 22.1, P < 0.001$	$F_{2,92} = 24.1, P < 0.001$
Ambient daily maximum mean temperature during incubation	$F_{2,57} = 1.91, P = 0.17,$ $R^2_{adj} = 0.04$	$F_{2,57} = 2.26, P = 0.14,$ $R^2_{adj} = 0.03$	$F_{2,92} = 3.66, P = 0.06,$ $R^2_{adj} = 0.19$	$F_{2,92} = 2.79, P = 0.09,$ $R^2_{adj} = 0.22$
Elevation	$F_{2,57} = 1.77, P = 0.18$	$F_{2,57} = 0.19, P = 0.66$	$F_{2,92} = 1.08, P = 0.30$	$F_{2,92} = 10.4, P < 0.01$
Ambient daily minimum mean temperature during incubation	$F_{2,57} = 0.92, P = 0.34,$ $R^2_{adj} = 0.02$	$F_{2,57} = 0.02, P = 0.88,$ $R^2_{adj} = -0.008$	$F_{2,92} = 0.91, P = 0.34,$ $R^2_{adj} = 0.16$	$F_{2,92} = 1.37, P = 0.24,$ $R^2_{adj} = 0.21$
Elevation	$F_{2,54} = 2.73, P = 0.10$	$F_{2,54} = 1.80, P = 0.19$	$F_{2,92} = 6.70, P < 0.05$	$F_{2,92} = 22.68, P < 0.001$
Ambient daily maximum mean temperature during posthatch	$F_{2,54} = 0.04, P = 0.83,$ $R^2_{adj} = 0.01$	$F_{2,54} = 0.07, P = 0.79,$ $R^2_{adj} = -0.002$	$F_{2,92} = 0.004, P = 0.94,$ $R^2_{adj} = 0.16$	$F_{2,92} = 0.15, P = 0.69,$ $R^2_{adj} = 0.20$
Elevation	$F_{2,54} = 0.007, P = 0.93$	$F_{2,54} = 1.80, P = 0.18$	$F_{2,92} = 3.70, P = 0.057$	$F_{2,92} = 13.9, P < 0.01$
Ambient daily minimum mean temperature during posthatch	$F_{2,54} = 0.24, P = 0.62,$ $R^2_{adj} = 0.01$	$F_{2,54} = 0.28, P = 0.59,$ $R^2_{adj} = 0.001$	$F_{2,92} = 0.07, P = 0.79,$ $R^2_{adj} = 0.0001$	$F_{2,92} = 0.81, P = 0.37,$ $R^2_{adj} = 0.21$

Significant *P* values are shown in bold.

Table S3

F statistics for models with reproductive parameters as response variables and elevation, daily maximum and daily minimum temperature means (incubation¹, posthatch² periods) as predictor variables

Model	Clutch size ¹	Brood size ²	Mass ²	Asymmetry ²
2018				
Elevation	$F_{3,52} = 2.39, P = 0.13$	$F_{3,51} = 2.73, P = 0.10$	$F_{3,51} = 0.26, P = 0.61$	$F_{3,51} = 6.05, P = 0.01$
Microclimate daily maximum mean temperature	$F_{3,52} = 0.024$	$F_{3,51} = 0.02, P = 0.88$	$F_{3,51} = 0.31, P = 0.58$	$F_{3,51} = 0.06, P = 0.80$
Nest depth	$F_{3,52} = 0.15, P = 0.70,$ $R^2_{adj} = -0.01$	$F_{3,51} = 0.002, P = 0.96,$ $R^2_{adj} = 0.005$	$F_{3,51} = 0.97, P = 0.33,$ $R^2_{adj} = -0.03$	$F_{3,51} = 0.08, P = 0.77,$ $R^2_{adj} = 0.08$
Elevation	$F_{3,52} = 0.0001, P = 0.99$	$F_{3,51} = 1.87, P = 0.18$	$F_{3,51} = 0.37, P = 0.54$	$F_{3,51} = 2.27, P = 0.28$
Microclimate daily minimum mean temperature	$F_{3,52} = 0.85, P = 0.36$	$F_{3,51} = 0.00, P = 0.99$	$F_{3,51} = 0.35, P = 0.56$	$F_{3,51} = 0.74, P = 0.39$
Nest depth	$F_{3,52} = 0.22, P = 0.63,$ $R^2_{adj} = 0.005$	$F_{3,51} = 0.001, P = 0.97,$ $R^2_{adj} = 0.004$	$F_{3,51} = 1.00, P = 0.32,$ $R^2_{adj} = -0.03$	$F_{3,51} = 0.04, P = 0.83,$ $R^2_{adj} = 0.09$
Elevation	$F_{3,52} = 2.57, P = 0.11$	$F_{3,52} = 3.91, P = 0.05$	$F_{3,52} = 0.02, P = 0.88$	$F_{3,52} = 6.81, P < 0.05$
Ambient daily maximum mean temperature	$F_{3,52} = 0.21, P = 0.65$	$F_{3,52} = 0.77, P = 0.38$	$F_{3,52} = 0.00, P = 0.98$	$F_{3,52} = 1.48, P = 0.23$
Nest depth	$F_{3,52} = 0.22, P = 0.64,$ $R^2_{adj} = -0.008$	$F_{3,52} = 0.003, P = 0.95,$ $R^2_{adj} = 0.03$	$F_{3,52} = 1.26, P = 0.27,$ $R^2_{adj} = -0.03$	$F_{3,52} = 0.10, P = 0.74,$ $R^2_{adj} = 0.1$
Elevation	$F_{3,52} = 1.16, P = 0.28$	$F_{3,52} = 1.51, P = 0.22$	$F_{3,52} = 0.41, P = 0.52$	$F_{3,52} = 0.39, P = 0.53$
Ambient daily minimum mean temperature	$F_{3,52} = 0.51, P = 0.48$	$F_{3,52} = 0.59, P = 0.44$	$F_{3,52} = 0.39, P = 0.53$	$F_{3,52} = 1.69, P = 0.20$
Nest depth	$F_{3,52} = 0.23, P = 0.63,$ $R^2_{adj} = -0.002$	$F_{3,52} = 0.002, P = 0.96,$ $R^2_{adj} = 0.03$	$F_{3,52} = 1.36, P = 0.25,$ $R^2_{adj} = -0.02$	$F_{3,52} = 0.07, P = 0.79,$ $R^2_{adj} = 0.03$
2019				
Elevation	$F_{3,91} = 0.56, P = 0.46$	$F_{3,91} = 2.33, P = 0.13$	$F_{3,91} = 0.46, P = 0.49$	$F_{3,91} = 0.33, P = 0.57$
Ambient daily maximum mean temperature	$F_{3,91} = 0.48, P = 0.49$	$F_{3,91} = 0.29, P = 0.59$	$F_{3,91} = 0.15, P = 0.70$	$F_{3,91} = 0.01, P = 0.91$
Nest depth	$F_{3,91} = 0.25, P = 0.61,$ $R^2_{adj} = -0.002$	$F_{3,91} = 0.06, P = 0.79,$ $R^2_{adj} = 0.002$	$F_{3,91} = 0.19, P = 0.66,$ $R^2_{adj} = -0.02$	$F_{3,91} = 0.94, P = 0.33,$ $R^2_{adj} = -0.02$
Elevation	$F_{3,91} = 0.07, P = 0.78$	$F_{3,91} = 0.09, P = 0.75$	$F_{3,91} = 0.04, P = 0.83$	$F_{3,91} = 0.18, P = 0.67$
Ambient daily minimum mean temperature	$F_{3,91} = 0.70, P = 0.40$	$F_{3,91} = 0.26, P = 0.61$	$F_{3,91} = 0.02, P = 0.89$	$F_{3,91} = 0.02, P = 0.89$
Nest depth	$F_{3,91} = 0.29, P = 0.59,$ $R^2_{adj} = 0.0002$	$F_{3,91} = 0.06, P = 0.80,$ $R^2_{adj} = 0.002$	$F_{3,91} = 0.19, P = 0.66,$ $R^2_{adj} = -0.02$	$F_{3,91} = 0.94, P = 0.33,$ $R^2_{adj} = -0.02$

Significant *P* values are shown in bold.

Supplementary figure legends**Fig. S1**

Mean (a) daily minimum and (b) daily maximum microclimate nestbox temperatures (\pm SE) during incubation and posthatch periods in 2018.

Fig. S2

Mean daily minimum ambient temperature (\pm SE) during nest-building, incubation and posthatch periods in (a) 2018 and (b) 2019.

Fig. S3

Mean daily maximum ambient temperature (\pm SE) during nest-building, incubation, and posthatch periods in (a) 2018 and (b) 2019.

Supplementary figures

Fig. S1

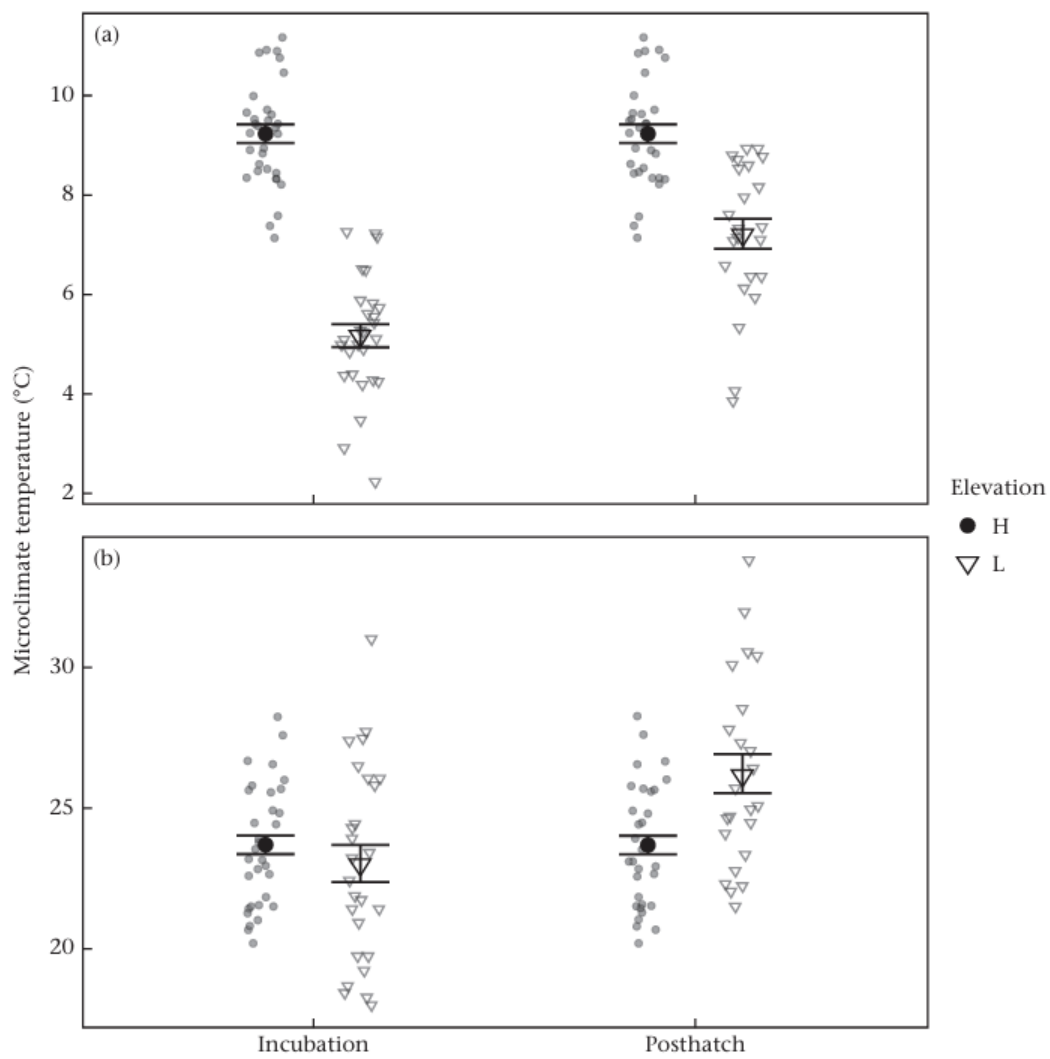


Fig. S2

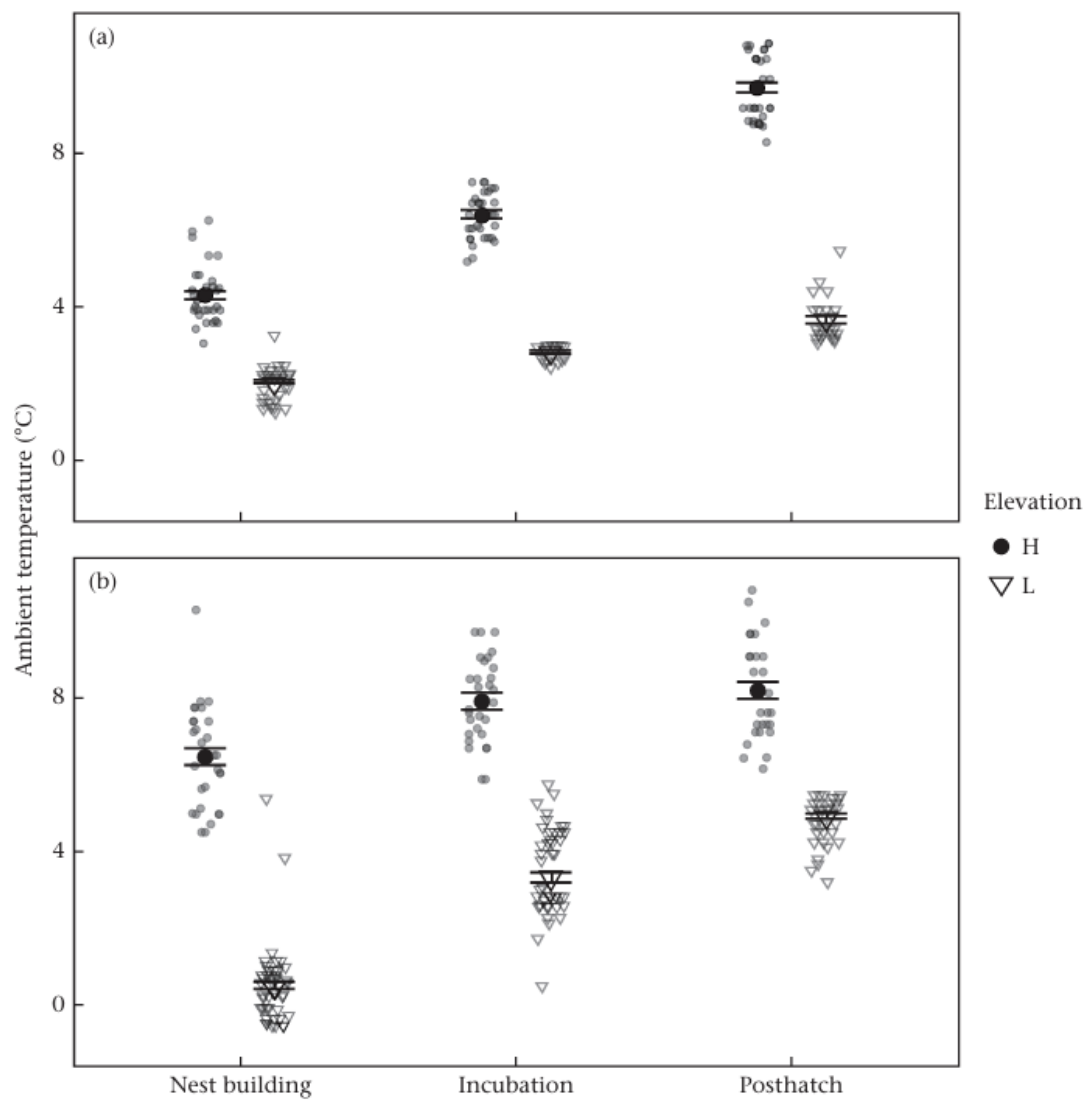
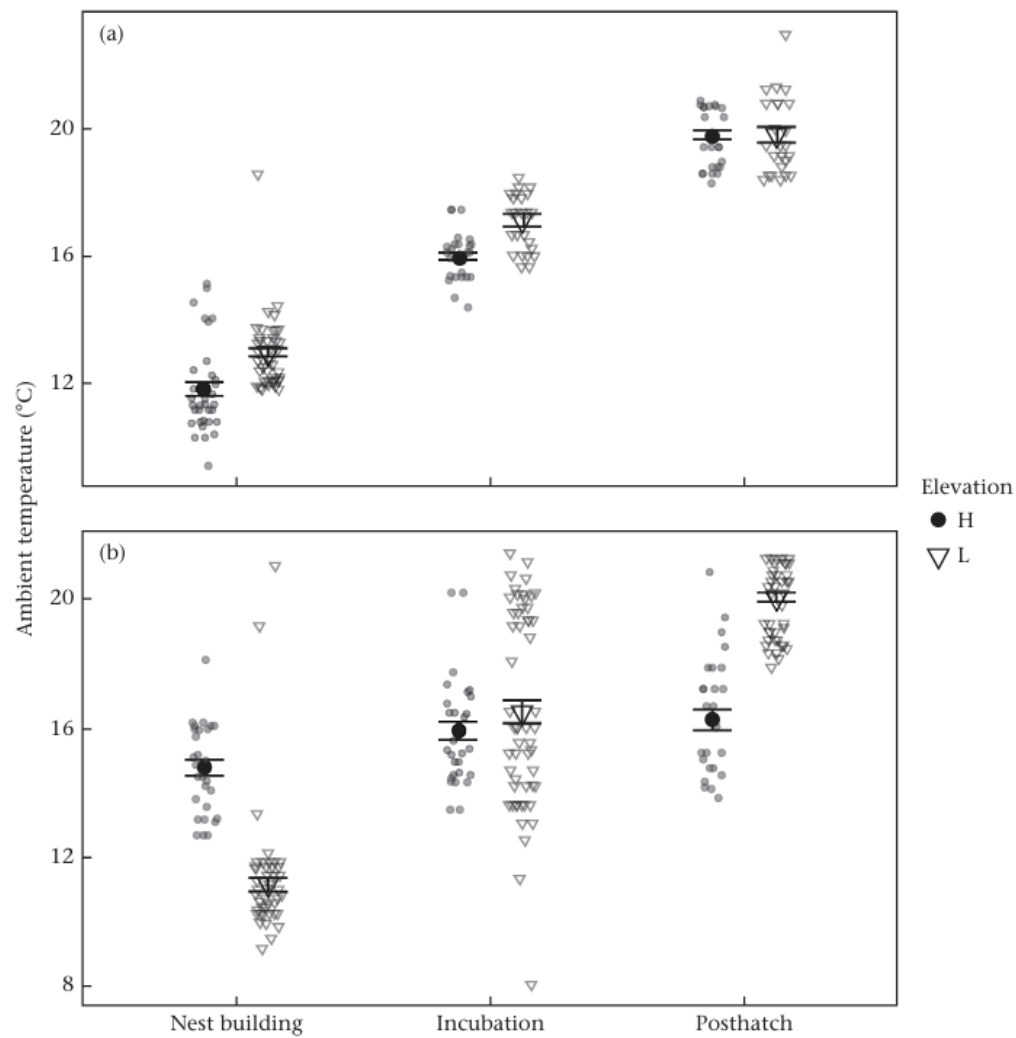


Fig. S3



Chapter 3

***Protocalliphora* prevalence is related to nest composition and elevation, but not to nestling fitness in a resident cavity-nesting bird**

Authors: Benjamin R. Sonnenberg^{1*}, Carrie L. Branch², Lauren M. Benedict¹,
Angela M. Pitera¹, Mike B. Teglas¹, and Vladimir V. Pravosudov¹

Institutions:

¹University of Nevada, Reno, Department of Biology, Ecology, Evolution and
Conservation

Biology Graduate program, Reno, NV, USA

²Department of Psychology, University of Western Ontario, London, ON, Canada

*Corresponding Author

Abstract

Understanding how the environment mediates host-parasite interactions is critical for predicting the future fitness of both hosts and parasites. Indeed, the local environment may be especially important for ectoparasites, with the abiotic and biotic conditions that surround the host and influence its behavior directly impacting the future fitness of both the host and the parasite. For example, a host may actively choose to reside in environments that reduce the negative fitness consequences of parasitic interactions, especially during key life history events such as reproduction. Avian species are well-studied regarding host-parasite interactions and display a number of behavioral defenses including physically removing parasites via preening and avoiding microhabitats with higher parasite densities. Here, we investigated the consequences of nest size and composition in relation to ectoparasite (*Protocalliphora*) load in resident mountain chickadees (*Poecile gambeli*) at two elevations in the Sierra Nevada mountains in North America. Higher elevations in montane coniferous forests in northern latitudes have harsher environments defined by high and persistent snow levels during the winter that shorten the reproductive window for resident birds. Nest composition, regardless of elevation, was highly repeatable within individual females across years. However, high elevation nests on average contained less animal hair and fewer ectoparasites compared to low elevation nests. Yet, there was no relationship between offspring immune activity (i.e., heterophile lymphocyte (H/L) ratio) or any other aspect of nestling condition and ectoparasite load, suggesting

that the naturally present parasite load in our study system did not have any measurable impact on offspring fitness while still in the nest.

Keywords: host-parasite interactions, nest composition, elevational gradient, ectoparasites, immune response, chickadee

Introduction

The physiological and behavioral defenses that free-living organisms mount in response to environmental challenges are diverse and often context specific (Lindström 1999, Bicudo et al. 2010, Sokolova 2021, Wani et al. 2022). These challenges can originate from abiotic factors, such as local climate, or from con- and heterospecific members of their communities (Clayton & Moore 1997, Lindström 1999, Wolinska & King 2009, Anderson & May 2022). The interactions between local environments, hosts, and the parasitic organisms that rely on hosts as necessary habitats to survive and proliferate result in some of the most well-studied host defenses (Anderson & Gordon 1982, Clayton & Moore 1997, Wolinska & King 2009, Penczykowski et al. 2016, Scholl et al. 2019, Anderson & May 2022).

The interaction dynamics within community structures can be mediated by direct interactions, such as predation or competition, or by indirect interactions driven by processes such as parasitism (Lehmann 1993, Sheldon & Verhulst 1996, Clayton & Moore 1997). Parasitism has been shown to impact a host's ability to mount immune responses (Sheldon & Verhulst 1996), compete for resources (Bedhomme et al. 2005, Friesen et al. 2017), and successfully reproduce (Read 1988, Zuk & Loya 1991, Bollache et al. 2001, Friesen et al. 2017). Avian hosts are exposed to a wide array of both ecto- and endoparasite species (Clayton & Moore, 1997, Samour, 2016). Some parasite species may last the entire lifespan of the avian host, while others may be relatively short-lived and regulated by a host's mounted immune response (Clayton & Moore 1997,

Dawson et al. 2005, Tschirren et al. 2007). Still others, such as larval *Protocalliphora*, are often only experienced for a specific time by an avian host during early development (i.e., in the nest) (Dawson et al., 2005, Johnson & Albrecht, 1993 O'Brien et al., 2001). Larval *Protocalliphora* are hematophagous parasites that hatch in the nest substrate and crawl onto their hosts to access blood meals (Whitworth & Bennett, 2011, Deeming & Reynolds 2015, Scholl et al. 2019).

Evidence for the impacts of hematophagous parasites on both nestlings and adult birds exposed during reproduction has been mixed, with some studies reporting strong negative effects while others report no detectable decrease in host fitness (Arendt et al. 1985, Roby et al. 1992, Johnson & Albrecht 1993, Lehmann 1993, Merino & Potti 1995, Clayton & Moore 1997, Tripet & Richner 1997, Szép & Møller 2000, Wesółowski 2001, Simon et al. 2004, O'Brien et al. 2001 & 2008, Dudaniec et al. 2006, Lobato et al. 2008, Streby et al. 2009). This may suggest that hosts have evolved a clear immune response and thus are only negatively impacted in years when high infestation rates are coupled with other challenges, such as inclement weather or poor resource availability (Roby et al. 1992, Richner & Heeb, 1995, Clayton & Moore 1997, Streby et al., 2009). The presence of an evolved immune response (i.e., ability to withstand negative parasite impact) is supported by the host response to a novel infestation on the Galapagos Islands, where multiple native avian species exhibited high mortality when first exposed to an introduced hematophagous parasite (Fessler & Tebbich 2002, Dudaniec et al., 2006, Fessler et al. 2018). This severe response was likely

resulting from a lack of co-evolutionary history between ectoparasite and host (Fessler et al. 2018).

The majority of avian species construct nests during reproduction, and these structures provide critical functions such as thermoregulatory assistance during incubation, securing eggs and offspring during development, and protection from predators (Ar & Sidis 2002, Deeming & Reynolds 2015). At the same time, these nests provide habitat for ectoparasites that give ideal access to incubating parents and developing offspring (Clayton & Moore 1997, Deeming & Reynolds 2015). Nest size and composition have been shown to impact both endo- and ectoparasite loads (Remeš & Krist 2005, Moreno et al. 2009, Reynolds et al. 2016, Ganser et al. 2020), but the intensity of these infestations is also mediated by temperature and humidity (Heeb et al. 2000, Cantarero et al. 2013). Thus, where and how individuals construct nests within their environment may have a strong effect on future parasite load (Remeš & Krist 2005, Reynolds et al. 2016).

The procurement and use of nest substrate containing high amounts of plant secondary metabolites has long been hypothesized to decrease offspring exposure to parasitism and increase fitness benefits for both the parents and offspring (Wimberger 1984, Clark & Mason 1985 & 1988, Clayton & Moore 1997, Petit et al. 2002, Mennerat et al. 2009, Tomás et al., 2012). Some experimental studies (i.e., researchers supplementing the nests with greenery such as yarrow) have shown this effect, but there is limited evidence for this being a natural behavioral defense (Petit et al. 2002, Shutler & Campbell 2007). Another study in

an urban innovator, the house sparrow (*Passer domesticus*), showed that nests that contained cigarette material had lower parasite loads, but this was likely due to material availability and not a host defense mechanism (Suárez-Rodríguez et al. 2013). Substrate use may depend on other factors, such as experience or social learning (Breen et al. 2021, Camacho-Alpizar et al. 2021, Whittaker et al. 2023). Additionally, animal material, such as mammalian hair, is used by many avian species for enhanced thermoregulatory abilities of the nest (Deeming & Reynolds 2015). However, using animal material has also been shown to increase parasite load in the nest (Blunsden & Goodenough, 2023). Thus, there may be a potential tradeoff in providing thermoregulation to nestlings during early development and exposure to larger parasite loads later in development.

For this study, we measured innate nestling immune activity (i.e., heterophile lymphocyte (H/L) ratio), nest composition, and ectoparasite load in a resident population of mountain chickadees (*Poecile gambeli*) in the Sierra Nevada mountains, USA. We studied a population at two elevations in a natural, relatively undisturbed, coniferous montane forest. Chickadees are obligate secondary cavity-nesting species that readily use provided nest boxes during reproduction (Sonnenberg et al. 2019, McCallum et al. 2020). Past work in this system has shown that chickadees build variably sized nests but that individual females build similar sized nests across years (Sonnenberg et al. 2019, Sonnenberg et al. 2022). Compositional differences between nests remains understudied, however, due to the homogenous structure of these montane forests with likely equal access to nest substrate type and likely small differences

in substrate use across elevations (Sonnenberg et al. 2019). However, although it is known that this species is exposed to ectoparasites in the nest, the impact of nest composition and how the chickadee hosts respond to this exposure remains unknown (Gold & Dahlsten 1983, McCallum et al. 2020).

Chickadees are highly resident and remain at high elevations throughout the year (McCallum et al. 2020). Individuals residing at higher elevations in the Sierra Nevada experience harsher winters, and this winter weather often carries over into the spring and dictates when some animals can initiate reproduction (Kozlovsky et al. 2018, Sonnenberg et al. 2020, Whitenack et al. 2023). However, how these cooler temperatures and persistent snow levels impact parasitism at high elevations is undescribed. There is mixed evidence for how elevation might impact parasite prevalence, with some species increasing in density (Moreno-Rueda 2021, González et al. 2014), others showing a decrease in prevalence (Zamora-Vilchis et al. 2012, González et al. 2014), while still others show no differences in parasite prevalence at all (González et al. 2014). Residing in high elevations with less predictable weather events, harsher overall climate, and higher demands on physiological function is known to select against the persistence of some animal populations, but whether this is the case for ectoparasites remains unclear (Bicudo et al. 2010).

We predicted that chickadee nests would show no compositional differences between elevations, but compared to high elevation, low elevation nests would have higher ectoparasite loads due to earlier breeding initiation timing and overall warmer temperatures (Sonnenberg et al. 2019, Whitenack et

al. 2023). Additionally, we predicted that compared to high elevation, low elevation nests would be associated with reduced fitness (i.e., smaller brood sizes, lower nestling mass, greater fluctuating asymmetry) and higher immune activity (i.e., H/L ratio) in response to these higher ectoparasite loads.

Methods

Site and species

All data for this study were collected during April–August (the chickadee reproductive period) of 2018 and 2019 at the Sagehen Experimental Forest (Sagehen Creek Field Station, Tahoe National Forest, University of California, Berkeley) near Truckee CA, USA. We monitored two subsets of the population, one at a high elevation transect, ‘high’ (range: 2380 m–2590 m; coordinates: 39.42402, -120.315015) and the second at a low elevation transect, ‘low’, (range: 1965 m–2070 m; coordinates: 39.443500, -120.243248) (Whitenack et al. 2023). Individual birds were trapped at feeders in the fall and winter months (August–March) using mist nests and were trapped by hand at nestboxes during the spring and summer months (May–July). Upon capture, birds were tagged with unique combinations of leg bands which include colored plastic leg bands, individually numbered aluminum bands band issued by the United States Geological Survey (USGS), and colored plastic leg bands equipped with passive integrated transponders (PIT) tags (Bridge et al. 2019). During the initial capture, all birds were aged and sexed if possible, using a combination of physiological

(sex: brood patch, cloacal protuberance) and morphological (age: feather structure and molt pattern) indicators (Pyle 2022).

Nest and reproductive monitoring

Approximately 350 nest boxes were maintained and monitored for the duration of the study (Kozlovsky et al. 2018, Sonnenberg et al. 2019, Whitenack et al. 2023). Beginning in the second week of April, boxes were checked several times a week for signs of nest-building, egg-laying, incubation, hatching, and nest success. The ordinal date for the first egg in each nest was recorded and clutch size (i.e., total number of eggs in the nest) was recorded after the female began incubating. The depth of each nest was measured and recorded at the start of incubation following the previously established protocol (Sonnenberg et al. 2019). Female chickadees incubate for approximately 13 days, and monitoring for incubation initiation allowed for nests to be closely checked for hatching and for hatch date to be precisely recorded for each nest. This is critical as nestlings were all processed on day 16 post hatch for accurate across-year comparison of nestling condition.

During processing, nestlings were removed from the nest and counted to ascertain brood size (i.e., total number of nestlings at day 16). Each nestling was then weighed on an electronic scale to the nearest 0.01 of a gram, banded with a USGS numbered leg band, a small blood sample was taken (primarily for multiple ongoing genetics studies), and both outer tail feathers were measured to the nearest 0.5 mm. The absolute value of the difference between these feather

lengths was used as a measure of fluctuating asymmetry (i.e., a proxy for developmental stress) (Palmer & Strobeck 1986, Eeva et al. 2000, Sillanpää et al. 2010). Clutch size, brood size, mean nestling mass (grams), fluctuating asymmetry, and the coefficient of variation of nestling mass within nests were used as estimates of parental reproductive output and offspring quality.

Nestling blood differential analysis

During initial nestling processing on day 16 post hatch, a blood sample was taken from each nestling. Approximately 10 μ L of blood tissue was used to make standard blood smears on glass microscope slides immediately after the sample was taken (Scanes & Dridi 2021, Samour, 2016). Each slide was dried in the field and fixed with methanol in the lab within eight hours. Slides from each bird were then dyed in small batches of approximately 10 slides each using a standard Romanowsky stain (e.g. Diff Quik) and air dried (Clark 2014, Samour 2016). Dried slides were then fixed with a permanent coverslip that allowed for multiple future analyses.

The number of two leukocyte cell types (heterophils and lymphocytes) were counted to a sum of 100 for each slide with a light microscope at 1000x magnification with oil immersion (Banbura et al. 2013, Clark 2015, Samour 2016). The number of heterophils and the number of lymphocytes were used to calculate a ratio (H/L) value for each nestling, and these were averaged across all nestlings from each unique nest. Values above 0.5 indicate a prolonged

exposure to elevated circulating glucocorticoids (Davis et. al 2008, Samour 2016).

The production of erythrocytes (red blood cells), or erythropoiesis, was estimated using the same slides (Glomski & Pica 2016). These counts were performed to measure estimates of blood generation and whether these estimates varied with parasite load. Avian red blood cells are nucleated, and young cells can be easily identified using a polychromatic index that relies on differentially colored nuclei (Glomski & Pica 2016 , Samour 2016). Erythrocytes were counted at 1000x magnification with oil immersion in groups of 100 for three separate fields of view on the same slide. These counts were then averaged and used to calculate the percentage of polychromatic cells for each individual (Glomski & Pica 2016, Samour 2016). These estimates were then averaged across all the nestlings from each unique nest.

We additionally screened slides for the presence of common endoparasites but were unable to detect a single specimen (Glomski & Pica 2016, Samour 2016). However, this result may be due to the age of the nestlings as they may not have been actively infected for a long enough time to exhibit detectable symptoms.

Nest dissection and parasite quantification

Chickadee nestlings fledge between day 22–24 post hatch, and nests were collected within one to two days after fledging was detected (McCallum et al. 2020). Nests were stored in gallon resealable plastic bags and placed in a

freezer until processing. Nests were composed primarily of a mix of mammal-derived hair and woody materials, and all nests were hand dissected using insect tweezers and kitchen strainers. Strainers were used to remove excess dust from the nest material and then the nests were separated into their basic material components. Mammalian hair was separated and weighed to the nearest 0.01 gram. The plant material components (mostly small pieces of pine woodchips) and the total mass of the nest were also weighed to the nearest 0.01 gram. Nests were checked for the presence or absence of owl pellet material and feathers (other than chickadee feathers) because these items appeared to be abundant and rare respectively. Initial observations suggested that chickadees may be forced to utilize previously regurgitated animal hair material in their nests due to a potential limitation of available animal hair in their environment. However, it is unlikely that these materials are unevenly distributed across elevations. Feathers were quite rarely detected and have been shown to be highly sought after in other species, and so their presence or absence was scored (Winkler 1993, Lombardo et al. 1995). We predicted that the nests of experienced individuals (those which had bred previously) would be more likely to contain such limited items (Pitera et al. 2021).

Lastly, during nest dissection we collected and counted the pupae or pupal cases of *Protocalliphora* that were present in each nest (Dawson et al. 2005). The majority of these cases were found embedded within the mammal hair found within each nest. Material was also examined for the presence of other

ectoparasite taxa but very few were discovered or identified and thus were omitted from analyses.

Statistical analyses

We used generalized linear models to test for relationships between nest composition, ectoparasite load, and offspring fitness including their immune response. For all analyses, we only included nests that were from the first breeding attempt of a given pair within a season, as second nesting attempts are rare in our system. We included the identification of the individual females in the majority of models as we had a large number of the same females present in both years of the study.

All models and associated figures were generated with R version 4.3.1 (Whickham et al. 2019, R Core Team 2024). Using the package 'glmmTMB', we fit linear mixed models to test the effects of elevation and year on aspects of nest size, composition, and parasite load (Brooks et al. 2017). In addition to elevation and year, ectoparasite load and multiple aspects of chickadee reproductive output were used as predictors to test for a relationship with offspring immune response (Lymphocyte ratio). If significant differences were detected between elevation or year, the package 'emmeans' was used to conduct post hoc analyses and acquire model corrected categorical means (Lenth 2021). We also used binomial regressions to test for the differences in the probability of owl pellet material and feather material occurring in the nest using the predictors year, elevation, and female experience. Lastly, we used the 'rptR' package to estimate repeatability scores (R) for aspects of nest composition including total

nest mass, mass of mammal hair, and mass of plant material in the nest using year and elevation as fixed effects and female identification as a random effect (Stoffel et al. 2017). We used a Gaussian distribution and parametric bootstrapping (nboot = 1500) to obtain 97% confidence intervals for the estimate.

All models were checked for normality and fit using the 'DHARMA' package (Hartig 2022).

Results

Nest size and composition across years and elevations

Nest mass was highly correlated with nest height (a metric used in a previous study within this system; Sonnenberg et al. 2019), and nest mass was smaller at high elevation compared to low elevation (post hoc: estimate = -5.96 ± 2.93 , df = 91, t ratio = -2.-3, p = 0.04; low elevation: n = 55, mean = 37 grams; high elevation: n = 50, mean = 32 grams) but nest mass did not change across the two years of study (Table 1; Model 1). Total nest mass, the total amount of mammal-derived hair within a nest, and the size of the nest base (plant material composition) were all significantly repeatable within individual females across years (Table 2). When analyzed separately, the mammalian hair component of an average nest was much larger (post hoc: estimate = -4.05 ± 0.90 , t ratio = -4.49, p < 0.001, n = 105) at low elevation (mean = 6.40 grams) compared to high elevation (mean = 2.35 grams) (Table 1: Model 2).

Nests of experienced vs non-experienced females were equally as likely to contain feathers regardless of year or elevation (Table 3). However, the low

elevation nests were more likely (65%) to contain owl pellet derived material compared to high elevation nests (39%) regardless of the experience of the nest builder or year of the study (Table 3).

Ectoparasite load, nest composition, and nestling immune response

Of all the nests that were surveyed, 89.5% contained ectoparasites. There were slight differences between elevations, with 86% of high elevation and 92.7% of low elevation nests containing parasites. Ectoparasite load (total number of parasites detected within each nest) was lower in high elevation nests compared to low elevation but did not change across years (Table 4, Fig. 1B). Additionally, nests that contained more mammal hair had higher numbers of ectoparasites in the nest even when controlling for brood size (Table 1, Fig. 2A). Despite differences in ectoparasite load, there was no relationship between ectoparasite load and nestling immune response (Table 4, Fig. 1A). There was also no relationship between multiple measures of reproductive output (i.e., first egg date, clutch size, nestling mass, coefficient of variation in nestling mass, and fluctuating asymmetry) and ectoparasite load, except for brood size: nests with more nestlings contained more parasites. However, no reproductive parameter was associated with nestling immune response (Table 5).

Nests with higher ectoparasite loads contained nestlings with higher polychromatic scores (response: polychromatic score; predictors: intercept = estimate: 18.02 ± 1.81 , $z = 9.94$, $p < 0.001$, $n = 40$; parasite number = estimate: 0.05 ± 0.02 , $z = 2.24$, $p = 0.025$; elevation = estimate: 6.10 ± 2.43 , $z = 2.51$, $p =$

0.012, Fig. 2B). This result suggests that nestlings were losing more blood in nests with more parasites, but that such loss still did not elicit a detectable immune response or a detectable decline in nestling mass. Differences in polychromatic score based on elevation was likely driven by higher parasite load at lower elevations (post hoc: estimate = -6.1 ± 2.43 , $df = 35$, t -ratio = -2.51 , $p = 0.017$, $n = 40$; high elevation: polychromatic score: 21 ± 1.7 , low elevation: polychromatic score: 27.1 ± 1.55).

Discussion

Our results showed that nests of mountain chickadees at low elevation had higher *Protocalliphora* numbers compared to high elevation nests and that these higher infestation rates corresponded to higher amounts of mammalian hair material in the nests. Overall, over 89% of nests within this system, regardless of year or elevation, showed parasitism, but we did not detect significant relationships between parasite load, nestling immune response, or any aspect of reproductive output measured in the population. Despite a lack of immune activity response, nestlings did show higher blood productivity in response to higher ectoparasite load. This evidence suggests that nestlings had to self-invest more during developmental time in nests with higher *Protocalliphora* loads, yet there were no detectable tradeoffs associated with nestling mass or immune activity in any measure used in this study.

Our results corroborate a plethora of past work showing that many species do not show strong responses to the presence of *Protocalliphora* infestations

during the nesting period (Johnson & Albrecht 1993, Lehmann 1993, Richner & Heeb, 1995, O'Brien et al. 2001, Simon et al. 2004, Streby et al. 2009). It is often suggested that potential tradeoffs in response to parasite load manifest after nestlings have fledged, and that nestlings during this post-fledging period are more likely to show detrimental effects of parasites or may even be less likely to survive (Brown et al. 1997, Streby et al. 2009). Our results that nestlings are producing more blood to compensate for parasite exposure show that there is some energetic cost, but we were not able to detect this cost impacting the nestlings' mass or immune response during the time in the nest (Simon et al. 2004, Carleton 2008).

It is unclear whether differences in ectoparasite prevalence by elevation simply correspond to nest composition or if *Protocalliphora* are not as abundant at higher elevations in the Sierra Nevada. It is likely that nest composition in mountain chickadees is restricted at higher elevations due to snow cover limiting females' access to potential materials during the nest building period (Kozlovsky et al. 2018, Sonnenberg et al. 2019, Whitenack et al. 2023). Past work in this system showed that females build nests of consistent depths, but it was unclear whether this was independent of nest composition (Sonnenberg 2019, 2022). This study confirms that not only was nest depth and overall nest mass repeatable within individual females, but that separate components of the nest materials are also repeatable. In addition, high elevation nests contained on average less mammal hair than low elevations nests. Work in a closely related European species has shown that nests comprised of more hair also contain

more parasite larva, matching the results of our study (Blundsden & Goodenough 2023). However, other studies have shown no association between the amount of hair and presence of parasites (Remeš & Krist, 2005, Cantarero et al. 2013, Blundsden & Goodenough 2023). Overall, it is possible that *Protocalliphora* show strong preferences for mammal hair and that a lack of mammalian hair led to lower levels of infestation at elevations where hair was a limited resource.

The repeatability of nest size and composition indicates that nest building behavior in chickadees has a strong innate component (Sonnenberg et al. 2019, 2022). Indeed, past work in this system demonstrated the strong relationship between the winter food-caching propensity of this species, a highly innate behavior with a genetic basis, and nest building behavior (Sonnenberg et al. 2022). Females that cached more food also built larger nests, forming a suite of correlated behaviors that may share an underlying physiological mechanism. With all evidence taken together, it is doubtful that females differentially construct nests with less hair or other materials in order to limit parasite exposure to their offspring. Given that there seems to be very little cost (i.e. detectable immune response, reduction in mass, etc.) for nestlings from ectoparasite exposure, there may be relaxed selection pressures on preferential nest characteristics (materials and size), allowing the highly adaptive and innate food-caching behavior to spillover and impact nest building in this species.

Past work in this system has shown that experienced parents produce offspring of higher quality (Pitera et al. 2021). However, nest composition was not related to female experience as certain limiting materials were not more or less

likely to be found in the nests of first-time versus experienced breeders. This likely means that females are not traveling great distances to seek out specific materials and that there is not significant competition for these materials. Our findings contrast with those reported for other species such as tree swallows, which compete over feathers to include in their nests (Winkler 1993, Lombardo et al. 1995).

Overall, our study adds to the current evidence that *Protocalliphora* have very little detectable fitness impact on developing avian young while in the nest, despite chickadee nestlings' long exposure to these parasites (approx. 22–24 days of development). There were also no detectable differences in immune response between years or between elevations at our study site despite drastic differences in environmental conditions including cooler temperatures and higher snow levels at higher elevations especially in years where winter conditions persisted into the reproductive period (i.e., following winters with above average precipitation). Our work also provides further evidence that chickadee nest building behavior is likely heritable as individuals build repeatable nests structures from year to year both in size, mass, and composition.

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Author contributions: Conceived the idea, design, experiment: BRS, VVP. Data collection: BRS, CLB, AMP, LMB. Wrote the paper: BRS (substantially edited the paper: VVP). Developed or designed the methods: BRS. Analyzed the data: BRS. Contributed funding: VVP, BRS, LMB

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Tables

Table 1: Nest size, mass, and composition				
<i>Fixed effect</i>	<i>N</i>	<i>Parameter estimate</i>	<i>z-value</i>	<i>p-value</i>
Response variable: Total nest mass				
Intercept	105	-12.09 ± 6.04	-2.00	p = 0.04
Nest depth		6.82 ± 0.89	7.69	p < 0.001
Elevation		5.96 ± 2.93	2.03	p = 0.04
Year		0.79 ± 2.02	0.39	p = 0.69
Mixed effect: Female identification: 55.95 ± 7.48; AIC: 768.3				
Response variable: mammalian hair material				
Intercept	104	1.38 ± 0.75	1.84	p = 0.06
Parasites/nestling		0.12 ± 0.06	2.05	p = 0.04
Elevation		3.30 ± 0.95	3.47	p < 0.001
Year		0.76 ± 0.78	0.97	p = 0.33
Mixed effect: Female identification: 6.43 ± 2.54; AIC: 601.6				

Table 2: Nest size and composition within-female repeatability			
<i>Repeatability estimate</i>	<i>SE</i>	<i>CI</i>	<i>p-value</i>
Total nest mass			
0.77	0.08	[0.59, 0.89]	p < 0.001
Mammal fur mass			
0.40	0.17	[0, 0.71]	p = 0.014
Plant material mass			
0.71	0.10	[0.49, 0.86]	p < 0.001

Table 3: Likelihood of composition use				
<i>Fixed effect</i>	<i>N</i>	<i>Parameter estimate</i>	<i>z-value</i>	<i>p-value</i>
Model 1: use of feathers				
Intercept	97	0.67 ± 0.66	1.00	p = 0.314
Experience		-0.41 ± 0.59	-0.70	p = 0.485
Elevation		0.28 ± 0.46	0.62	p = 0.534
Year		-0.41 ± 0.46	-0.89	p = 0.371
Mixed effect: Female identification: 0.06 ± 0.24; AIC: 120.9				
Model 2: use of owl pellet				
Intercept	97	-0.53 ± 0.67	-0.78	p = 0.432
Experience		0.10 ± 0.62	0.17	p = 0.867
Elevation		2.04 ± 0.68	2.97	p < 0.01
Year		-0.03 ± 0.50	-0.07	p = 0.943
Mixed effect: Female identification: 0.46 ± 0.68; AIC: 121.2				

Table 4: Immune response, ectoparasites, and reproductive output				
<i>Fixed effect</i>	<i>N</i>	<i>Parameter estimate</i>	<i>z-value</i>	<i>p-value</i>
Model 1: Immune response (Leukocyte ratio) ~ parasite load				
Intercept	40	0.97 ± 0.16	6.10	p < 0.001
Ectoparasite load		-0.01 ± 0.06	-0.10	p = 0.92
Elevation		0.04 ± 0.17	0.25	p = 0.80
Year		-0.02 ± 0.16	-0.13	p = 0.89
Mixed effect: Female identification: 0.04 ± 0.19; AIC: 67.0				
Model 2: Immune response (Leukocyte ratio) ~ brood size				
Intercept	40	1.13 ± 0.34	3.30	p < 0.001
Brood size		-0.02 ± 0.05	-0.48	p = 0.63
Elevation		0.04 ± 0.15	0.25	p = 0.80
Year		-0.03 ± 0.15	-0.19	p = 0.85
Mixed effect: Female identification: 1.1e ⁻⁸ ± 1.0e ⁻⁴ ; AIC: 66.9				
Model 3: Immune response (Leukocyte ratio) ~ mean nestling mass				
Intercept	40	1.32 ± 1.74	0.76	p = 0.45
Nestling mean mass		-0.03 ± 0.14	-0.21	p = 0.84
Elevation		0.04 ± 0.16	0.23	p = 0.82
Year		-0.02 ± 0.15	-0.13	p = 0.89
Mixed effect: Female identification: 0.04 ± 0.21; AIC: 67.0				
Model 4: Immune response (Leukocyte ratio) ~ coefficient of variation in nestling mass (CV)				
Intercept	40	1.30 ± 0.25	5.14	p < 0.001
CV		-6.00 ± 3.75	-1.60	p = 0.11
Elevation		0.01 ± 0.15	0.06	p = 0.95
Year		-0.03 ± 0.15	-0.23	p = 0.82
Mixed effect: Female identification: 0.02 ± 0.14; AIC: 64.6				
Model 5: Immune response (Leukocyte ratio) ~ mean fluctuating asymmetry (FA)				
Intercept	40	1.19 ± 0.19	6.08	p < 0.001
FA		-0.41 ± 0.23	-1.77	p = 0.08
Elevation		0.14 ± 0.17	0.81	p = 0.42
Year		-0.05 ± 0.14	-0.36	p = 0.72
Mixed effect: Female identification: 0.13 ± 0.36; AIC: 63.4				

Table 5: Parasite load and reproductive parameters				
<i>Fixed effect</i>	<i>N</i>	<i>Parameter estimate</i>	<i>z-value</i>	<i>p-value</i>
Model 1: Parasite number ~ breeding timing				
Intercept	104	-80.18 ± 121.35	-0.66	p = 0.51
First egg date (ordinal)		0.68 ± 0.80	0.85	p = 0.39
Elevation		47.22 ± 14.23	3.32	p < 0.001
Year		13.64 ± 8.59	1.59	p = 0.11
Mixed effect: Female identification: 18.18 ± 4.26; AIC: 1081.1				
Model 2: Parasite number ~ clutch size				
Intercept	104	-15.83 ± 28.14	-0.56	p = 0.58
Clutch size		5.41 ± 3.83	1.41	p = 0.16
Elevation		37.90 ± 8.12	4.67	p < 0.001
Year		18.20 ± 8.34	2.18	p = 0.03
Mixed effect: Female identification: 9.83 ± 3.14; AIC: 1079.9				
Model 3: Parasite number ~ brood size				
Intercept	104	-17.34 ± 18.65	-0.93	p = 0.35
Brood size		6.51 ± 2.80	2.33	p = 0.02
Elevation		36.22 ± 8.04	4.52	p < 0.001
Year		17.64 ± 7.98	2.21	p = 0.03
Mixed effect: Female identification: 54.28 ± 7.37; AIC: 1076.5				
Model 4: Parasite number ~ mean nestling mass				
Intercept	104	89.76 ± 89.10	1.01	p = 0.31
Mean mass (g)		-5.38 ± 7.17	-0.75	p = 0.45
Elevation		36.48 ± 8.26	4.42	p < 0.001
Year		16.22 ± 8.21	1.97	p = 0.05
Mixed effect: Female identification: 46.54 ± 6.82; AIC: 1081.3				
Model 5: Parasite number ~ coefficient of variation in nestling mass (CV)				
Intercept	104	17.93 ± 11.40	1.57	p = 0.11
CV		90.48 ± 163.14	0.56	p = 0.58
Elevation		37.44 ± 8.15	4.59	p < 0.001
Year		15.27 ± 8.19	1.86	p = 0.06
Mixed effect: Female identification: 1.74e ⁻¹¹ ± 4.17e ⁻⁶ ; AIC: 1081.5				
Model 6: Parasite number ~ fluctuating asymmetry				
Intercept	104	13.15 ± 10.31	1.28	p = 0.20
FA (mm)		16.00 ± 13.38	1.20	p = 0.23
Elevation		31.24 ± 8.49	3.68	p < 0.001
Year		18.87 ± 8.13	2.32	p = 0.02
Mixed effect: Female identification: 5.25e ⁻⁵ ± 0.01; AIC: 1046.4				

Figure legend**Fig. 1**

Mean differences in variation by elevation in (A) immune activity (heterophil/lymphocyte (H/L) ratio) and (B) total *Protocalliphora* count with 95% confidence intervals.

Fig. 2

The linear relationships between the total amount of animal hair in the nest and parasite load (A) and parasite load and the regeneration of red blood cells (erythrocytes) by percentage of new cells present (B).

Figures

Fig. 1

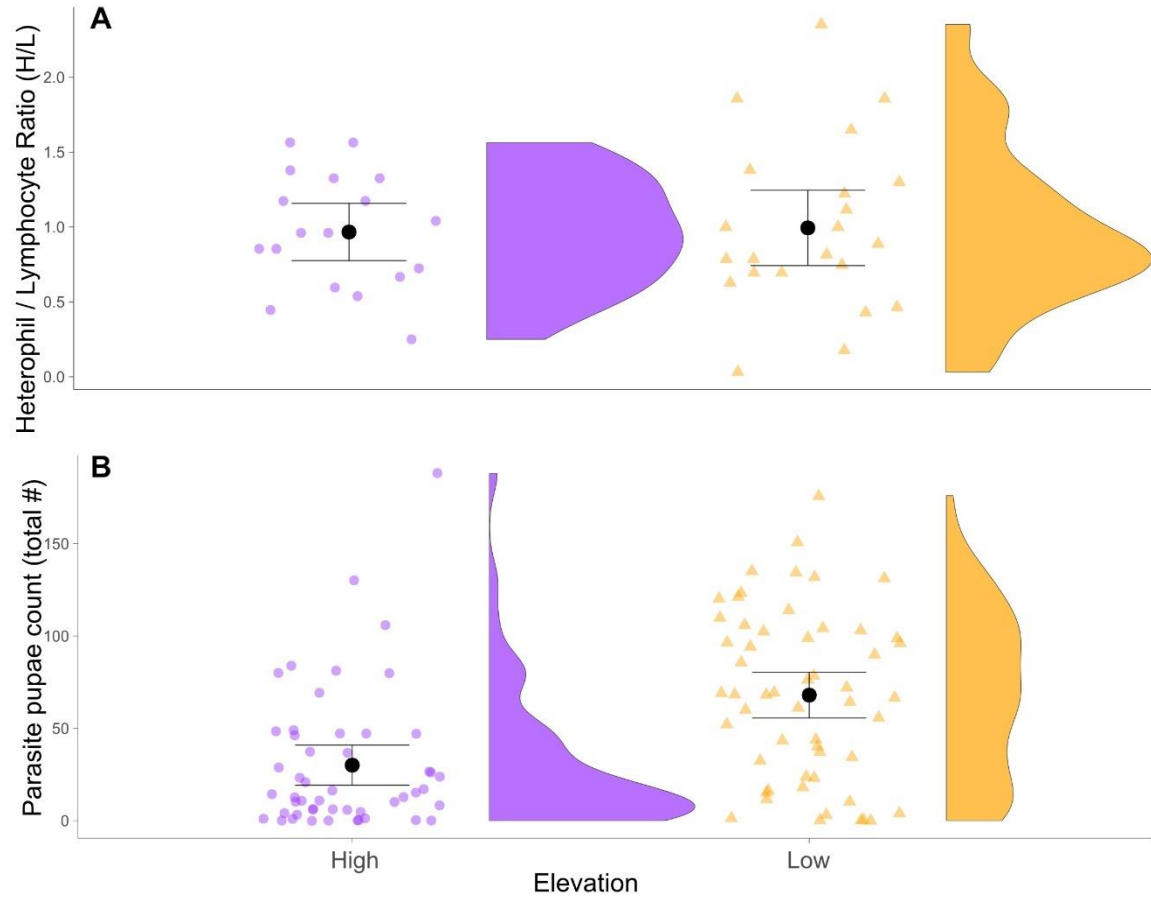
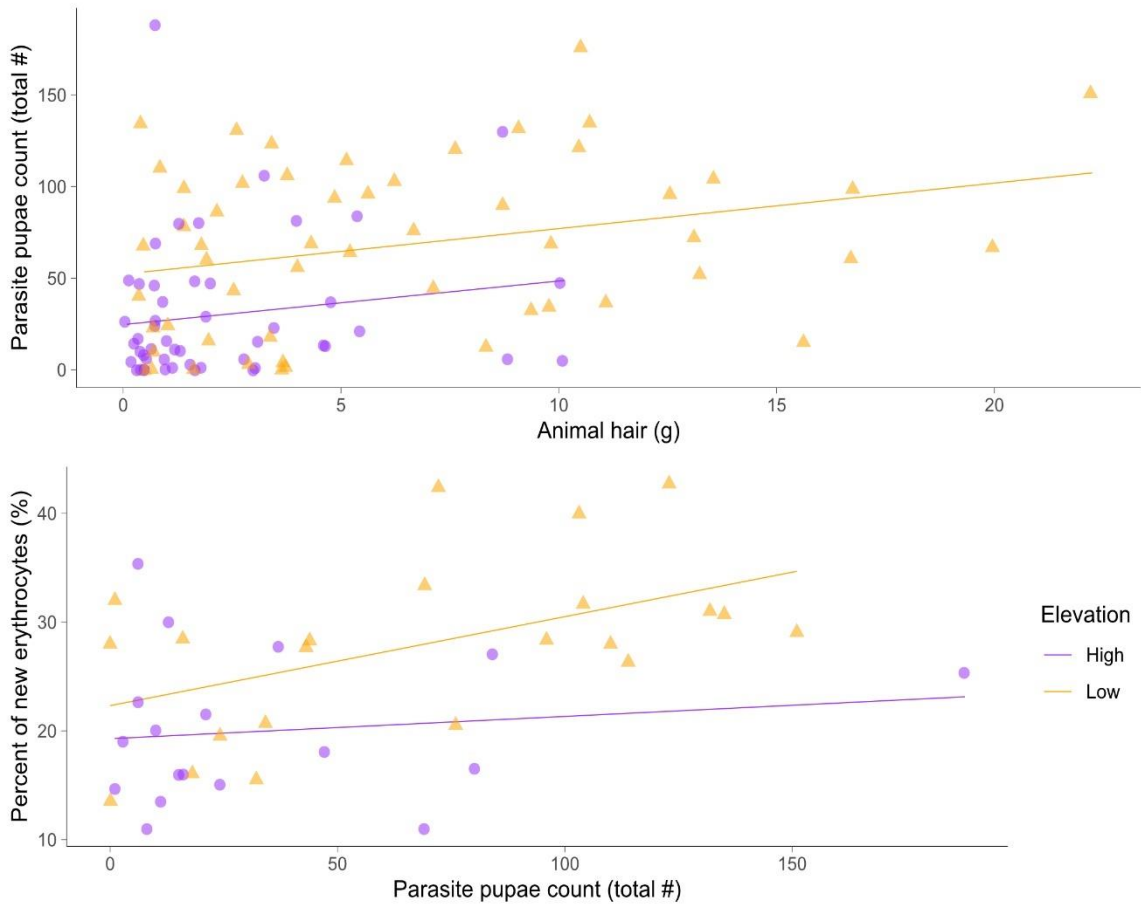


Fig. 2



Chapter 4

Food-hoarding and nest-building propensities are associated in a cavity-nesting bird

Authors: *¹Benjamin R. Sonnenberg, ²Carrie L. Branch, ¹Angela M. Pitera, ¹Lauren M. Benedict, ¹Virginia K. Heinen, and ¹Vladimir V. Pravosudov

Institutions:

¹University of Nevada, Reno, Department of Biology, Ecology, Evolution and Conservation

Biology Graduate program, Reno, NV, USA

²Cornell Lab of Ornithology, Cornell University, Ithaca, NY, USA

*Corresponding Author

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Abstract

The underlying mechanisms connecting correlated behaviors in wild populations remain largely unknown. Food-caching behavior is a prime example of an adaptive, compulsive-like behavior with a strong underlying innate drive—it starts after early development and is critical for survival—and individuals of some species rigorously and continuously cache up to tens of thousands of individual food items each season. Another behavior whose base processes may share similar underlying innate drive is nest building, as it involves repeatedly bringing material to the nest site often in a fixed pattern. There are various hypotheses attempting to explain inter- and intra-specific variation in nest characteristics, traditionally considering fitness-related consequences of such variation. Apparent non-functional nest variation remains largely unexplored but may have an association with other innately driven behaviors unrelated to nest building but potentially associated via a shared mechanism, such as food caching. Here, we show that individual variation in food hoarding is associated with differences in nest size in mountain chickadees (*Poecile gambeli*): individuals that cache more food also build bigger nests. Both behaviors are highly repeatable within individual females, but variation in nest size does not seem to have fitness consequences in our system. This finding suggests a possible connection in which the properties of one adaptive behavior may spillover and influence the outcome of another more neutral behavior, likely controlled by the same general underlying mechanism.

Keywords: food hoarding, nest building, behavioral syndrome, compulsive behaviors, chickadee

Introduction

Patterns of within-individual behavior that remain consistent across complex varying environments are of great interest to ecology and evolution (Sih et al. 2004a, b, Uher 2011). These suites of correlated behaviors, behavioral syndromes, are often composed of multiple repeatable behaviors that share a similar underlying drive (i.e., boldness; aggression) (Sih et al. 2004a, b, van Oers et al. 2004). There are a growing number of examples of such syndromes observed across wild systems (Sih et al. 2004a). Despite this, the underlying mechanisms and evolutionary implications of these behaviors remain largely unexplored (Sih et al. 2004a, b). A consequence of individuals exhibiting consistent behaviors across heterogeneous situations is that (i.e., bold or shy) individuals may experience context-dependent tradeoffs (Sih et al. 2004b; van Oers et al. 2004). How such tradeoffs are mediated on a mechanistic level is largely unknown (Sih et al. 2004a, b; van Oers et al. 2004). Additionally, whether one or more of the focus behaviors is under strong selective pressure may shape the interaction, both on a mechanistic and evolutionary scale, of the behaviors within a suite (Sih et al. 2004a, b).

One behavior that is critical to survival and repeated across years in highly variable contexts is food caching in non-human animals (Vander Wall 1990). Food caching appears to have evolved as an adaptation for sedentary species residing in relatively harsh and variable environments (Vander Wall 1990). Several avian species are well-known scatter-hoarders (e.g., Corvids, Parids, Picids), where individual birds may store tens or even hundreds of thousands of

individual food items during late summer and throughout the fall for later consumption (Pravosudov 1985, Vander Wall 1990, Pravosudov & Roth 2013). Individuals rely on these caches when food is scarce for overwinter survival (Vander Wall 1990, Clayton 1992, Clayton & Dickinson 1999, Pravosudov & Clayton 2002, Pravosudov & Roth 2013, Sonnenberg et al. 2019) and in some cases (e.g., in Canada and Siberian jays) for reproduction (Derbyshire et al. 2019, Sutton et al. 2019). The underlying motivational drive of long-term caching behavior appears highly rigid and compulsive-like—individuals start caching soon after becoming independent (Pravosudov 2006) and will, during the appropriate season, continue caching available food until supplies are depleted (Pravosudov & Roth 2013). Some species (e.g., Parids, Corvids, and Picids) will even cache non-food items, such as stones, in captive conditions once food resources are depleted (Kilham 1963, Clayton 1992, Clayton et al. 1994, Bugnyar et al. 2007) or begin caching early in development before retrieval behavior initiates (Suddendorf and Busby 2003).

Caching drive varies across species, populations, and environments, with some species showing sensitivity to pilfering, resource type, presence of conspecifics, and body condition (Lucas et al. 1993, Clayton et al. 2005, de Kort et al. 2007, Pravosudov 2008). However, Parids appear to be especially driven to cache food and will cache every few minutes throughout the day over the entire duration of the long-term caching period (Pravosudov 1985, 2006), caching double the amount they need to consume to survive the winter (Pravosudov 1985). In addition, food-caching Parids, unlike some Corvids, do not reduce their

caching rates in different social settings (i.e., when observed by potential pilferers) (Pravosudov 2008, Pravosudov et al. 2010) and actually increase their caching rates when caches were experimentally pilfered (Lucas et al. 2001). There is indirect evidence that baseline motivation to cache is heritable as individuals from harsher winter climates cache significantly more under controlled lab conditions compared to those from milder environments (Pravosudov & Clayton 2002, Freas et al. 2012), and these differences remained present when birds were hand-reared in identical captive environments (Roth et al. 2012). Altogether, these data strongly suggest that the baseline drive to cache can be described as compulsive. The result of such strong compulsive drive is the caching of much more than food than is necessary for survival (Pravosudov 1985) which is likely highly adaptive. Moreover, there is no evidence that this overcaching behavior constitutes long term future planning in caching species (Suddendorf & Corballis 2007). Harsh winter environments may frequently create conditions when some cache locations may not be accessible (e.g., snow storms) or disappear entirely (e.g., tree falls, deep snow). These same conditions may cause short-term variation in caching intensity, but the overall drive likely remains. Creating many more caches than is needed can provide better assurance against risk of starvation—and in small-bodied birds, risk of starvation is high (Pravosudov 2006). Evolving strong, compulsive-like drive to cache appears to be an effective solution to this problem.

Small Parid species such as mountain chickadees (*Poecile gambeli*) are well-known for exhaustively caching available resources during the fall months

when food items naturally used for caching (e.g., seeds of various pine species) are abundant (Haftorn 1992, Pravosudov 1985, Clayton 1992, Croston et al. 2016). Birds will also visit supplementary feeders hundreds to thousands of times a day in addition to natural sources to retrieve items to cache (Croston et al., 2016). However, caching rates of naturally available food peak in the fall, regardless of supplementation (Pravosudov 2006). The sheer number of detected daily caches and the peak of caching, specifically during the periods of over abundant food, suggests that caching drive is mostly independent from hunger state and may be more dependent on innate impulse, as it is impossible for these small birds to consume all these items in a 24-h period (Clayton 1992, Pravosudov 2006, Croston et al. 2016). Additionally, these food caches are generally consumed weeks and months after the date in which they were made, furthering this assumption (Vander Wall 1990, Pravosudov & Roth 2013). There is, however, no evidence that caches are recovered and used across years (Vander Wall 1990). Caches are a reliable food source and are retrieved in order to cope with uncertain climatic conditions over the winter months (Vander Wall 1990). Mountain chickadees residing in harsh environments, where winter conditions persist for longer periods and are more extreme, appear to require more food stores to survive compared to populations from milder areas (Pravosudov & Roth 2013, Croston et al. 2016, Sonnenberg et al. 2019). Chickadees from such environments have been shown to cache more both in the wild and in captive laboratory settings, reflecting this need (Freas et al. 2012, Croston et al. 2016).

Nest building has long been suspected of being controlled purely by innate drive and fixed genetic mechanisms (Mainwaring et al. 2014, Hall et al. 2015, Anholt 2020), though recent studies show the effect of experience in some species (Walsh et al. 2011, 2013, Breen et al. 2016, Camacho Alpizar et al. 2021). Often, the process of nest construction requires the collection and subsequent incorporation of hundreds of items into a cohesive structure and could potentially be under the influence of a strong innate drive with genetic underpinnings, as it involves highly repetitive actions throughout the nest-building period (Mainwaring et al. 2014, Anholt 2020). In fact, nest building in mice has been shown to have a strong genetic basis, and inbred lines can be selected for excessive nest-building behavior, which is characterized in part by highly repetitive, compulsive-like material gathering (Greene-Schloesser et al. 2011, Mitra & Bult-Ito 2021). The size of a nest approximately reflects the total number of gathering trips. In some ways, collecting nest materials resembles food caching, as individuals make repeated trips to gather and store materials. There is a plethora of evidence suggesting that both intra- and inter specific variation in nest structure and composition may also be related to local climate and experience (Britt and Deeming 2011, Crossman et al. 2011, Mainwaring et al. 2014, Camacho-Alpizar et al. 2021). However, some differences in nest structures appear to have little to no apparent fitness consequences, as demonstrated in mountain chickadees (Sonnenberg et al. 2020). For example, large differences in nest depth (which is a direct reflection of the overall nest size associated with the amount of material female birds brought to the nestbox) are

not associated with reproductive outcome or local climate across years with drastically different conditions (Sonnenberg et al. 2020).

Such seemingly non-functional variation in nest size suggests a possible link between unrelated behaviors that could have a similar underlying innate mechanism of control in which highly adaptive behaviors may spill over into other fit ness-neutral behaviors associated with the same mechanism. Different behaviors resulting from similar underlying mechanisms could directly influence each other, especially if one is under strong selection while the other is not. For example, strong selection for an increased food-caching drive may spillover to nest building and result in an explanation of nest size that remains unresolved by environmental variation during the breeding months (Sonnenberg et al. 2019, 2020). We addressed this question via the association between food caching propensity and nest size within individuals at our long-term field system in the northern Sierra Nevada, USA (Kozlovsky et al. 2018).

Previously, we found that individual female mountain chickadees built consistently sized nests across years, but that the population showed large variation in nest size (as measured by nest depth in standard-sized nestboxes), both within and across elevations that was not explained by local climate and resulted in no detectable fitness differences (Sonnenberg et al. 2020). As such, we hypothesize that food-caching and nest-building behaviors might be associated through a partially shared innate mechanism involving compulsive-like behaviors. Here, we (a) investigated trait (caching propensity and nest size)

repeatability and (b) compared winter food-caching propensity with nest size in females across years.

Methods

Subjects and site

All data for this study were collected 2016–2020 at Sagehen Experimental Forest (Sagehen Creek Field Station, University of California, Berkeley) in Sierra Nevada, USA, where we have studied individually marked (color bands and passive integrated transponder (PIT)-tags) mountain chickadees since 2014 (Kozlovsky et al. 2018) at two elevation sites, referred to as high (ca. 2400 m) and low (ca. 1900 m). It was not possible to record data blind because our study involved focal animals in the field.

Food-caching propensity

We used feeder visitation rates over the 4-day annual spatial cognitive testing task as a proxy for caching propensity (Croston et al. 2016). During such testing, PIT-tagged birds are expected to learn a location of a single rewarding feeder within an 8-feeder spatial array (Croston et al. 2016). The spatial array is equipped with modular microcontrollers connected to antennae built into the feeder perches that monitor all visitation (Croston et al. 2016). During testing, we counted the number of trials each bird completes over the 4-day task. A trial starts when a bird visits any feeder in the array and ends when it visits the correct rewarding feeder (Croston et al. 2016). Chickadees obtain one seed per trial and then fly away from the array with the seed to consume or cache it; as

such, one trial is equal to one seed collected from the feeder array. Chickadees complete hundreds of trials during the 4 days of testing; therefore, the number of trials is a good representation of food-caching propensity as a chickadee needs only a small fraction of these seeds to fulfill its metabolic requirements and most of the collected seeds are likely cached (Pravosudov 1985, Croston et al. 2016). A small portion of observed differences in visitation may be related to necessary food consumption; however, such differences appear to be relatively minor at this scale. Therefore, we used the total number of trials completed during the 4-day task as a proxy for caching propensity. Data across 5 years of testing was used for this study and was collected during winter months (2015-2016: November 30, 2016-2017: March 8th, 2017–2018: March 30, 2018–2019: April 8 and 2019–2020: January 20).

Nest measurements

Approximately 350 identically sized nest boxes (Sonnenberg et al. 2020) were monitored from April to July during 3 years of the study (2018–2020) across both elevations. Monitoring included checking each nest box for the status of nest construction, egg number, and hatch date during the nest-building, egg-laying, incubation, and post-hatch periods. Nest boxes were checked approximately one to two times a week. We used nest depth to estimate nest size, as it captures the approximate material amount deposited in each box, by measuring the depth at each of the four corners of the nest box with a metric ruler and then computing the mean of those values (Sonnenberg et al. 2020). Females are the sole nest builders in this species and so nest measurements

reflect an individual female's behavior. Mountain chickadees rarely lay two clutches of eggs in a year and so only first of the season nests were measured and included in analyses. All nests were measured after the onset of incubation which marks the end of nest building in mountain chickadees (Sonnenberg et al. 2020).

Statistical analyses

All data were analyzed using R statistical software (R v.4.0.3, R Core Team 2020). For all analyses, we combined females from high and low elevations as we were specifically interested in individual rather than elevation level variation of these behaviors and their potential associations.

Repeatable behaviors

The repeatability of both nest depth and caching propensity in individual females was calculated using the R package "rptR" (Stoffel et al. 2017). Repeatability scores were calculated using a Gaussian distribution and parametric boot strapping (nboot = 1500) to obtain 95% confidence intervals for each estimate. Repeatability estimates were calculated for females that participated in at least 2 years of the study. A total of 83 females were included in the analysis with 52 females detected in two nesting years and 31 females detected in all 3 years. Of these females, a total of 15 participated in spatial cognitive testing in more than 1 year (sampled across 2 years: n = 10, 3 years: n = 5). A second repeatability estimate for caching propensity was calculated for the entire participating population of chickadees across 5 years of comparable

study. A total of 183 individuals participated in two or more years of testing (sampled across 2 years: $n = 109$, 3 years $n = 47$, 4 years $n = 12$, 5 years $n = 10$).

Nest building and caching

To determine the relationship between nest depth and food caching propensity, mean nest depth was used as the dependent variable and mean total number of trials completed over the 4-day cognitive task served as the independent variable in a simple linear regression. There was a total of 57 females included in the model and the metrics of females that were detected in more than 1 year were averaged across years (1 year: $n = 42$, 2 years: $n = 10$, 3 years: $n = 5$). As 74% of all included females only had data for both caching and nest size for a single year, we averaged the data across the years for the remaining 26% as a more complex model seemed inappropriate (Silk et al. 2020). The data met all assumptions for the linear regression and images were created using ggplot2 (Wickham 2016, Zuur and Ieno 2016).

Results

Repeatability of nest size and food caching

Nest depth was significantly repeatable across 3 years in females ($n = 83$, $R = 0.629$, $SE = 0.064$, $CI = [0.494, 0.736]$, $p < 0.001$, Fig. 1). These results were consistent with our previously published estimates (Sonnenberg et al. 2020: ($R = 0.58$; $CI: 0.36, 0.73$; $p < 0.001$)).

Food-caching propensity, measured by the total number of trials completed during the 4-day cognitive task, was also significantly repeatable within individual females across years ($n = 15$, $R = 0.541$, $SE = 0.177$, $CI =$

[0.135, 0.83], $p = 0.006$, Fig. 2). Caching propensity was also found to have significant repeatability for the entire tested population across 5 years ($n = 183$, $R = 0.303$, $SE = 0.055$, $CI = [0.196, 0.413]$, $p < 0.001$, Fig. 3).

Nest size and food caching

The mean nest depth was significantly and positively associated with food-caching propensity across the 3 years of study ($n = 57$, $\beta = 0.35$, $F_{1,55} = 7.76$, adjusted $R^2 = 0.11$, $p = 0.007$, Fig. 4).

Discussion

Overall, our data support the hypothesis that food caching and some aspects of nest-building propensity are positively associated in mountain chickadees. Females exhibited high repeatability in both nest size and food-caching propensity across 3 years, despite marked differences in local climate between years (Kozlovsky et al. 2018, Sonnenberg et al. 2020). Furthermore, females that cached more food tended to build bigger nests (e.g., brought more nest materials).

Our findings suggest that variation in nest size may be linked to differences in food-caching motivation, likely as mechanistic spillover from a highly adaptive behavior to a potentially neutral one. We previously reported that variation in nest size was not associated with differences in female breeding experience, local climate, or reproductive success, but was decidedly consistent across years within individual females (Sonnenberg et al. 2020). If there is a similar underlying mechanism relating caching and some aspects of nest building, the behavior currently under stronger selective pressure (e.g., caching)

may be influencing the outcome of the other (e.g., nest size). In fact, there may be other nesting characteristics more important to offspring fitness that are unrelated to nest size, allowing nest size to be influenced by another trait. For example, nest site selection or increased female attentiveness while on the nest may be more significant to offspring fitness than nest size in obligate secondary cavity-nesting species (Bouvier et al. 2014, Sofaer et al. 2020, Sonnenberg et al. 2020). Thus, nest size has the potential of being indirectly modified by another behavior that is closely related through a shared mechanism without causing negative fitness consequences. In fact, the majority of evidence showing local climate's impact on nest structure in closely related taxa concerned non-caching species that may lack this underlying mechanistic connection to caching (Britt & Deeming 2011, Deeming et al. 2012). An alternative explanation is that increased food-caching propensity may be associated with improved individual condition prior to the breeding period, which may result in such individuals being more able to construct larger nests. We do not think this is a viable explanation for the findings presented here as we previously showed that large individual variation in nest size across different years was not associated with annual climatic variation and lacked clear fitness consequences (Sonnenberg et al. 2020). This result held true for both experienced females and first year breeding females (Sonnenberg et al. 2020).

The idea of spillover between seemingly unrelated behaviors is not uncommon (Sih et al. 2004b, Johnson & Sih 2005). Behavioral syndromes have been hypothesized to be genetically correlated which can imply a similar mech

anism of control (Sih et al. 2004a, b, Bell et al. 2009). Highly repetitive behaviors, such as food caching, can be described as compulsive-like as they are rigidly repeated, ritualistic, and often exceed the maximum fitness requirements. Such behaviors have an overabundance of underlying innate drive that may be decoupled from daily motivational state. This high level of baseline motivational drive may be a byproduct of selection, as for some traits, the repetitive nature of the behavior is highly beneficial to survival. If two behaviors (i.e., nest building and caching) share a mechanistic connection, the high baseline drive may spillover from one behavior into the other. There are numerous other compulsive-like behaviors exhibited in wild systems that incur fitness benefits (Eilam et al. 2006). For example, territory maintenance in a number of mammal species, in which they systematically retrace territory boundaries and repeatedly mark specific locations, comes with fitness benefits, including increased mate choice and decreased predation risk (Eilam et al. 2006). These repeated, ritualistic behaviors could easily be described as compulsive and benefit the performer through the maintenance of a large territory for foraging, mate attraction, and reproduction (Eilam et al. 2006). It also links two behaviors, locomotion and scent marking, which appear quite different mechanistically (Eilam et al. 2006). However, there are examples of how compulsive spillover can lead to less adaptive outcomes. For example, the aggressive spillover hypothesis proposed in several arachnid species provides an explanation as to why female spiders may kill and eat potential mates despite costly fitness consequences (Johnson 2013). Apparent compulsive female aggression overwhelms courtship behavior,

resulting in either driving the male away or consuming the male before copulation can take place (Johnson 2013).

Our results also provide an excellent provocation for future work into the underlying mechanisms relating correlated behaviors. For example, one would assume that if the multiple behaviors in a suite are mechanistically related, then that could affect the outcome of one by directly manipulating the other. This may not be the case for this particular example as these behaviors are temporally separated (aka: caching takes place in the fall and winter months, where nest building takes place in the spring) and altering caching behavior by manipulating the immediate environment (e.g., physical or social environment) may not directly impact the shared mechanism controlling the overall caching motivation. However, it may be possible in future to precisely manipulate the underlying baseline drive, by either increasing or decreasing the innate motivational state. As this study only provides the very preliminary description of this syndrome, future experimental work of this nature would be required in order to actually tease apart the causal connection between nest building and caching.

Overall, we provide novel data linking two behaviors with different functions, food caching and nest building. These data suggest that one adaptive compulsive behavior, food caching, has the potential to affect, or spillover, into other unrelated behaviors. It remains possible that the association between food-caching and nest-building propensities found in our study are simply due to chance; however, this finding warrants more investigation. More data are needed to test if other disparate behaviors may be similarly associated with food-caching

drive. Additionally, our results may provide a means to investigate the mechanistic underpinnings of detrimental compulsive behaviors in humans, such as obsessive–compulsive disorder, using food-caching species as a model.

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Author contributions

BRS and VVP developed the ideas. BRS, CMB, AMP, LMP, and VKH participated in data collection. VKH, AMP, and VVP processed RFID data from cognitive testing; BRS analyzed the data. All authors co-wrote the manuscript.

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Ethics approval

This research has followed all the guidelines provided by the Institutional Animal Care and Use Committee of the University of Nevada, Reno (Protocol No. 00603), as well as any federal guidelines (California Department of Fish and Wildlife Permit D-0011776516-4).

Conflict of Interest

Authors declare no conflict of interest.

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Figure Legends

Fig. 1

Nest depths in individual females across 3 years

Fig. 2

Total trial number (caching propensity) of individual females across 3 years

Fig. 3

Total trial number (caching propensity) of individual birds across 5 years

Fig. 4

Relationship between the mean nest size (measured by nest depth (cm)) and food-caching propensity (measured by the mean number of trials completed across 4 days of spatial cognitive testing) in 57 female chickadees. Shaded area represents 95% confidence interval

Fig. 1

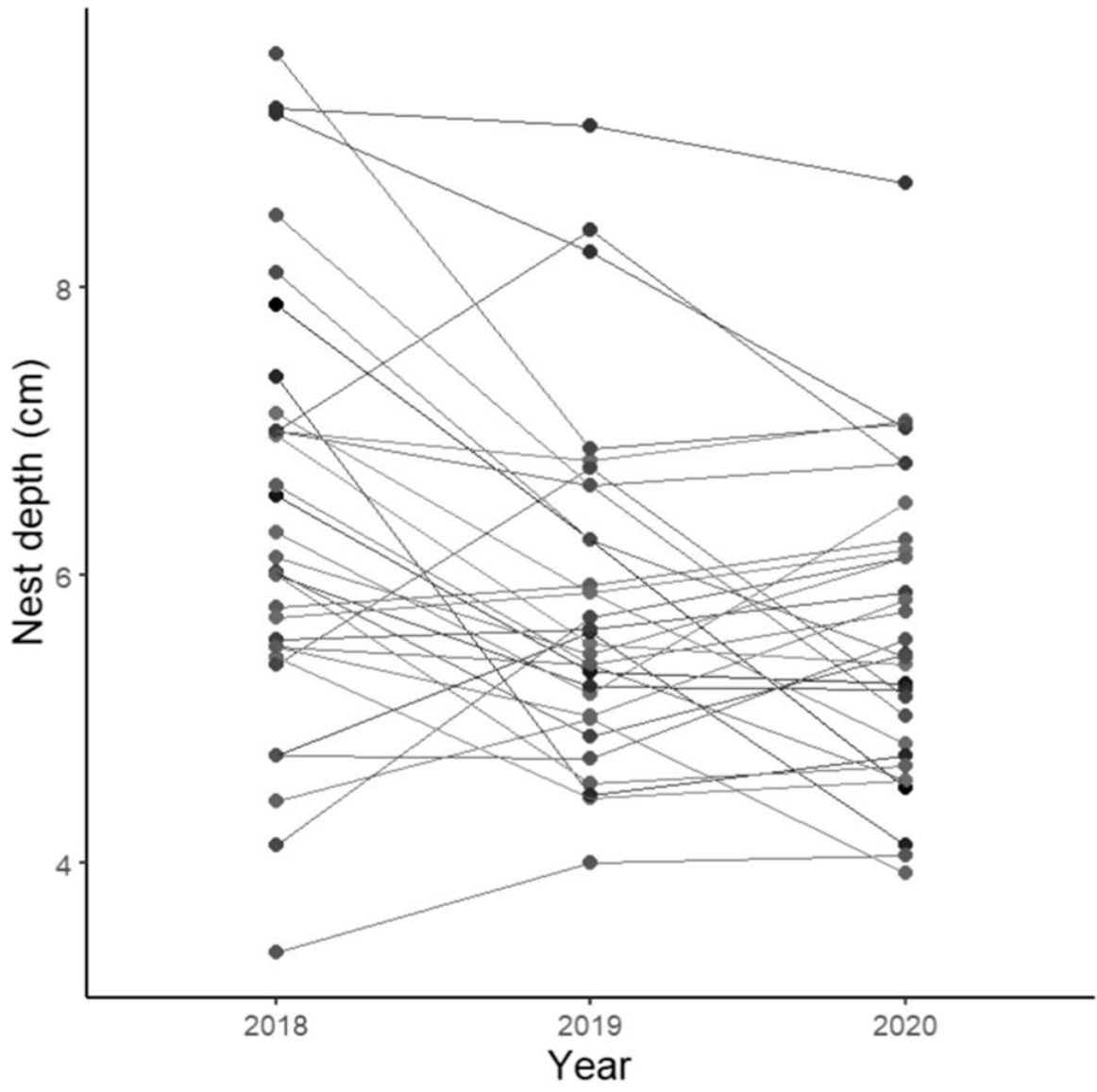


Fig. 2

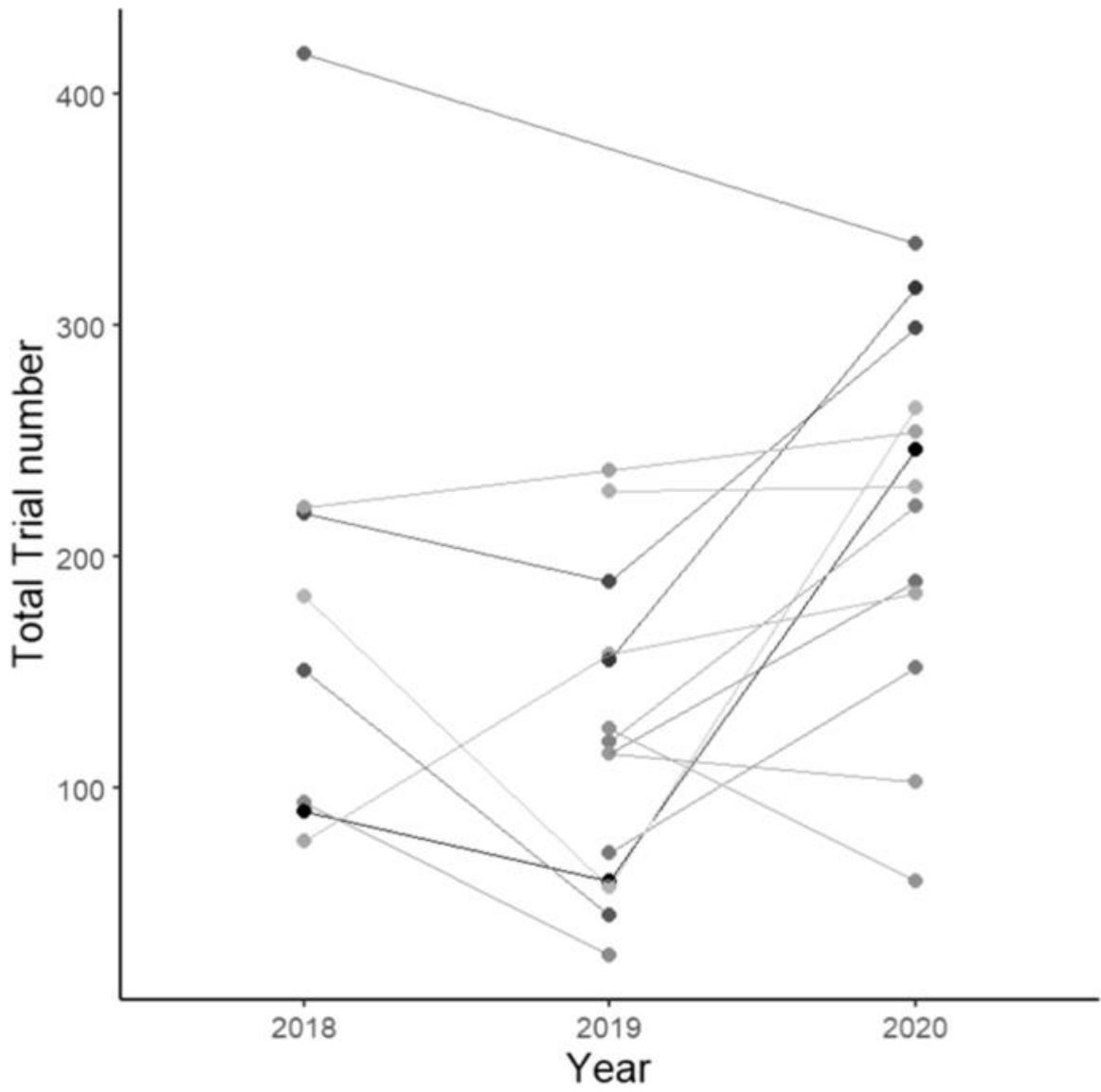


Fig. 3

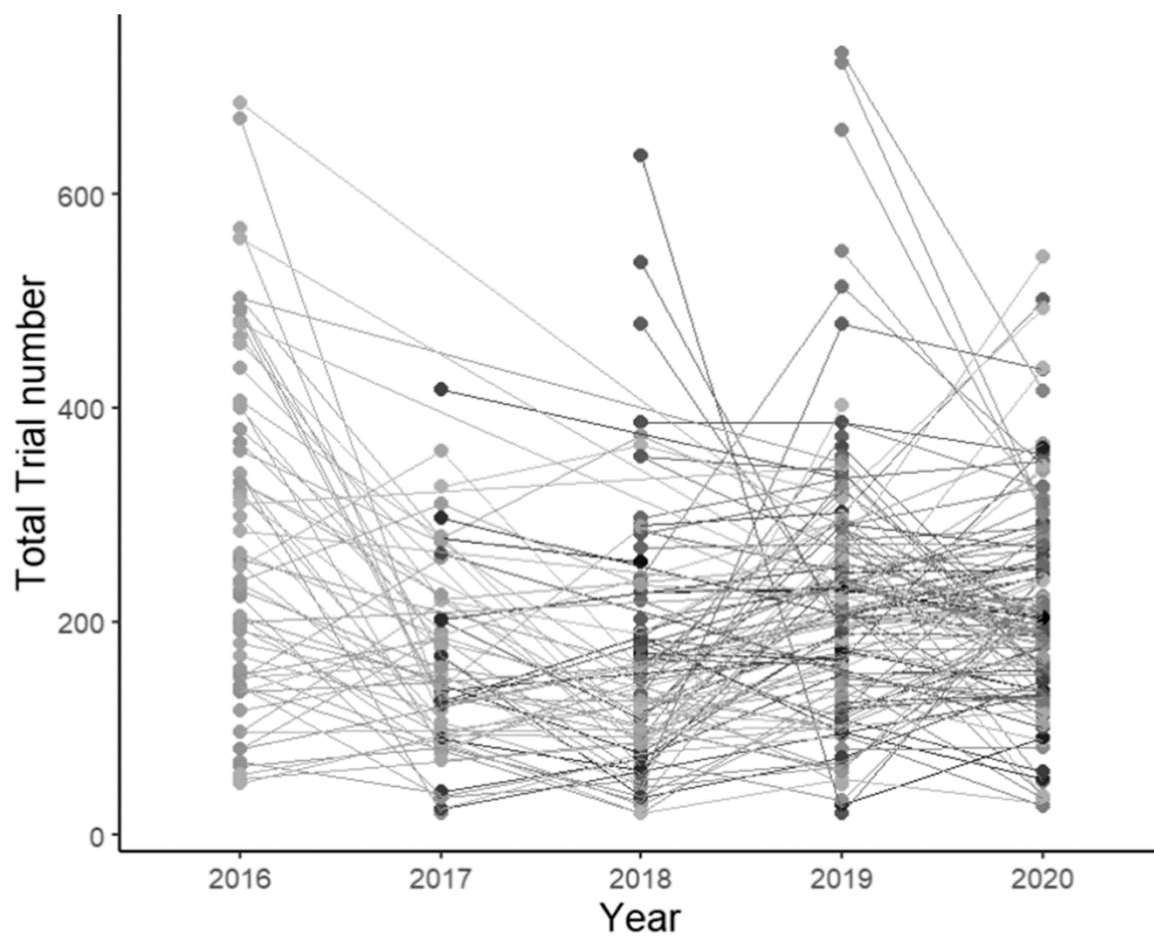
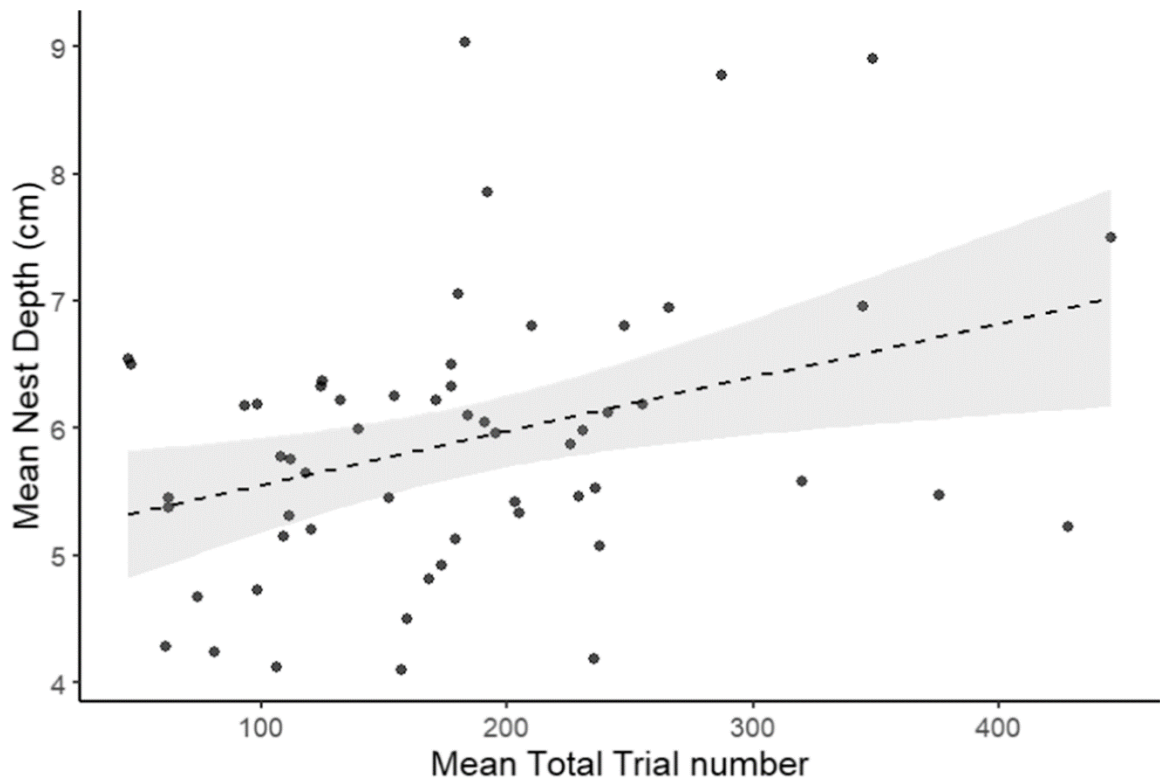


Fig. 4



Chapter 5

Natural variation in developmental condition has limited effect on spatial cognition in a wild food-caching bird

Benjamin R. Sonnenberg^{1*}, Virginia K. Heinen¹, Angela M. Pitera¹, Lauren M. Benedict¹, Carrie L. Branch², Eli S. Bridge³, Jenny Q. Ouyang¹, Vladimir V. Pravosudov¹

¹University of Nevada Reno, Department of Biology and Ecology, Evolution, and Conservation Biology Graduate Program, Reno, NV, USA

²Department of Psychology, University of Western Ontario, London, ON, Canada

³University of Oklahoma, Norman, OK, USA

*Corresponding author

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Abstract

Laboratory studies show that increased physiological burden during development results in cognitive impairment. In the wild, animals experience a wide range of developmental conditions, and it is critical to understand how variation in such conditions affects cognitive abilities later in life, especially in species that strongly depend on such abilities for survival. We tested whether variation in developmental condition is associated with differences in spatial cognitive abilities in wild food-caching mountain chickadees. Using tail feathers grown during development in juvenile birds, we measured feather corticosterone (Cort_f) levels and growth rates and tested these birds during their first winter on two spatial learning tasks. In only 1 of the 3 years, higher feather Cort_f was negatively associated with memory acquisition. No significant associations between feather Cort_f and any other measurement of spatial cognition were detected in the other 2 years of the study or between feather growth rate and any measurement of cognition during the entire study. Our results suggest that in the wild, naturally existing variation in developmental condition has only a limited effect on spatial cognitive abilities, at least in a food-caching species. This suggests that there may be compensatory mechanisms to buffer specialized cognitive abilities against developmental perturbations .

Keywords: developmental burden, cognition, memory, food-caching, chickadee, paridae

Introduction

Animals exhibit large variation, both across and within species, in cognitive abilities, yet the mechanisms underlying such variation remain poorly understood (Shettleworth 2009, Buchanan et al. 2013, Boogert et al. 2018). While selection can act on heritable traits associated with genetic differences (Croston et al. 2015, Branch et al. 2022), it remains uncertain how much individual variation in cognitive abilities observed in non-human animal populations may be produced by other factors, most notably differences in development (Hedges & Woon 2011, Schoech et al. 2011, Buchanan et al. 2013, Croston et al. 2015, Naninck et al. 2015, Boogert et al. 2018). Most research on animal cognition takes place in laboratory environments, which allows for the precise control of environmental conditions during both cognitive testing and development (Shettleworth 2009). Past laboratory-based work has shown that increased exposure to stress-inducing events during early development can have profoundly negative impacts on the future cognition of young animals (McEwen 2002, Pravosudov et al. 2005, 2006, Buchanan et al. 2013, Naninck et al. 2015). Cognitive traits depend on energetically expensive, specialized tissues (i.e. brain regions) to properly function and non-optimal conditions during development diverts energy expenditure towards short-term survival rather than neural investment (Pravosudov et al. 2005, Shettleworth 2009, Schoech et al. 2011, Naninck et al. 2015). The hippocampus, a brain region involved in spatial navigation and cognitive function, is known to be a major target of stress-

mediated effects, which appears to be driven by elevation in glucocorticoid hormones (Sapolsky et al. 1990, McEwen 2002, Hedges & Woon 2011).

Glucocorticoids are a well-studied class of steroid hormone that are known to mediate broad metabolic processes (Romero 2004, McEwen & Wingfield 2010, Vitousek et al. 2019, Guidi et al. 2021). Prolonged exposure to elevated levels of these hormones (namely cortisol and corticosterone) has been shown to increase the overall wear and tear on organisms over time (McEwen 2002, McEwen & Wingfield 2010, Guidi et al. 2021). Although these processes are regarded as normal signs of ageing, when they occur in young animals, they can severely alter developmental trajectories and appear to have a direct, negative impact on cognitive abilities in general (McEwen & Wingfield 2010, Hedges & Woon 2011, Schoech et al. 2011, Eyck et al. 2019), and particularly on hippocampus-dependent spatial abilities (Pravosudov et al. 2005, Naninck et al. 2015).

The ability to navigate through and remember past environments is a ubiquitous trait in animals. However, some species rely on the hippocampus for more specialized spatial cognitive abilities, for example, food-caching species (Shettleworth 2009, Pravosudov & Roth 2013). Scatter caching animals store thousands of individual food items throughout their environments and rely on spatial memory for future cache retrieval (Shettleworth 2009, Pravosudov & Roth 2013). In some North American chickadee species (*Poecile atricapillus* & *Poecile gambeli*), the dependence on food caches for overwinter survival has been

shown to vary across environments that differ in climatic harshness (Roth et al. 2012, Pravosudov & Roth 2013, Branch et al. 2019a, Sonnenberg et al. 2019). This environmental variation has also been linked with differences in spatial learning and memory ability and associated hippocampal morphology (Freas et al. 2012, 2013, Roth et al. 2012, Pravosudov & Roth 2013). Moreover, individual variation in spatial cognitive abilities in food-caching species appears to be associated with differences in overwinter survival (Sonnenberg et al. 2019) and has a genetic basis (Branch et al. 2022). This suggests that differences in spatial cognitive abilities in food-caching species probably arose via natural selection (Sonnenberg et al. 2019).

Considering that spatial cognitive abilities are critical for survival in food-caching species, it has been suggested that selection should favour the evolution of mechanisms that may protect them and their associated neural tissue from environmental perturbations during development (Buchanan et al. 2013). Yet, laboratory-based studies still show that increased developmental stress associated with impacts from malnutrition and prolonged exposure to elevated glucocorticoid levels, sometimes directly related to food deficits, result in impaired spatial cognitive abilities in some food-caching species (Pravosudov et al. 2005, Pravosudov & Kitaysky 2006). However, laboratory conditions provide highly impoverished environments compared to natural settings and may potentially restrict animals' abilities to resist or cope with developmental stress (Buchanan et al. 2013). Such restrictions include limited use of cognition (i.e. decreased opportunities to learn and practice) as well as rather restricted and

researcher determined nutrition (i.e. limited food diversity). Currently, little is known about whether developmental stress is strongly associated with impaired spatial cognitive abilities in wild animals living in their natural environment (Boogert et al. 2018).

In this study, we investigated whether natural variation in developmental conditions in juvenile birds was associated with differences in their spatial cognitive abilities. This study took place across three years using three separate cohorts of first-year wild mountain chickadees (*P. gambeli*). Mountain chickadees are small, highly sedentary songbirds that rely on specialized spatial cognition associated with food-caching to survive the montane winter environments in temperate western North America (Pravosudov & Roth 2013, Sonnenberg et al. 2019, McCallum et al. 2020, Branch et al. 2022). Birds residing at higher elevations that experience longer harsher winters compared to lower elevations appear to generally have better spatial learning and memory abilities (Freas et al. 2012, Croston et al. 2016) associated with a larger hippocampus and more hippocampal neurons (Freas et al. 2012). Additionally, strong directional selection favouring first-year birds with better spatial learning and memory performance was detected in an ongoing long-term study (Sonnenberg et al. 2019).

The condition of individual birds during post-hatching development was evaluated via differences in glucocorticoid hormone levels (corticosterone or Cort_t) (Bortolotti et al. 2008, 2009) and growth rates (Grubb 2006) measured in tail feathers. Malnutrition is known to result in slower feather growth rates and

elevated corticosterone levels (e.g. (Grubb 2006, Pravosudov et al. 2005, Pravosudov & Kitaysky)). Chickadees grow their first plumage while in the nest environment and before becoming nutritionally independent (McCallum et al. 2020). The quality of each feather, which is indicative of overall developmental conditions, is reflective of both the local environment and parental investment (Grubb 2006, Bortolotti et al. 2008, 2009, Will et al. 2014, McCallum et al. 2020, Jenni-Eiermann et al. 2022). Unlike measurements using blood samples, feather $Cort_f$ represents conditions during the entire early developmental period including time in the nest and the early post-fledging period, when tail feathers continue to grow (Bortolotti et al. 2008, 2009, Johns et al. 2018, McCallum et al. 2020). However, this method does come with some limitations including limited temporal understanding of when the hormone deposition took place and it is used here as a relative measure (Bortolotti et al. 2008, 2009).

Tail feather daily growth rates were assessed using feather growth bars (horizontal bands along the length of the feather), which accurately reflect caloric intake during a 24 h period (Grubb 2006). We predicted that patterns of yearly variation in individual developmental conditions would align with patterns of reproductive success in the larger population (i.e. years with smaller broods or lower nestling mass would also show more $Cort_f$). We collected tail feathers from juvenile chickadees throughout the autumn and winter and then tested their cognitive abilities in the same winter using two spatial cognitive tasks: a spatial learning and memory task and a single spatial reversal learning task (flexibility) following our well-established methods (Croston et al. 2016, 2017, Tello-Ramos

et al. 2018, Sonnenberg et al. 2019, Heinen et al. 2021). We predicted that chickadees that experienced worse developmental conditions (higher amounts of Cort_f deposited in feathers across development and smaller, slower growing feathers) would perform worse in both cognitive tasks compared to individuals with less Cort_f exposure and larger, faster growing feathers.

Methods

Study site

All data for this study were collected during 2018–2021 at the Sagehen Experimental Forest (Sagehen Creek Field Station, University of California, Berkeley) in the Sierra Nevada, USA. We monitor mountain chickadees year-round and collect annual measurements on reproductive success [36] and spatial cognitive performance (Croston et al. 2016, 2017, Tello-Ramos et al. 2018, Branch et al. 2019a, Sonnenberg et al. 2019, Heinen et al. 2021). We focus on two subsets of the population at two montane elevations, a high elevation site (approx. 2400 m) and a low elevation site (approx. 1900 m), which differ greatly in winter environmental conditions (Kozlovsky et al. 2018). High elevations are associated with harsher winters characterized by lower temperatures, higher and longer lasting snow levels and unpredictable winter storms compared to lower elevations (Heinen et al. 2021). We track individually marked birds across years via unique combinations of plastic colour bands, a passive integrated transponder (PIT) tag and in some cases an aluminium US Geological Survey band. We capture chickadees via mist net during the autumn and winter (non-

breeding), and at nestboxes during spring and summer (breeding), for banding. Nestlings are banded with only an aluminium leg band at day 16 post-hatch and, if recaptured, are then equipped with a PIT tag and an additional colour band. All birds are classified as juveniles (less than a year of age) or adults (more than a year of age) at time of initial capture based on multiple plumage characteristics (Pyle 1997), but only juveniles were included in this study.

Spatial cognitive testing

Across three years (2018–2021), we tested juvenile chickadees at both elevations using two spatial cognitive tasks—a spatial learning and memory task and a single spatial reversal learning task (Croston et al. 2016, 2017, Tello-Ramos et al. 2018). For cognitive testing, we used four feeder arrays, each composed of eight radio frequency identification (RFID) based feeders positioned equidistant on a square frame (122× 122 cm) and suspended ca. 4 m above the ground (Croston et al. 2016, 2017, Tello-Ramos et al. 2018, Sonnenberg et al. 2019). Two spatial arrays were used at each elevation, positioned approximately 1.5 km apart (Croston et al. 2016, 2017). Each RFID-equipped feeder contained an antenna embedded in a perch that was linked to an RFID-enabled Arduino circuit board with built-in memory and time keeping (Pyle 1997). This setup allowed us to record all visits by PIT-tagged birds (identification and visitation timing) and operate a motor-controlled door that managed food access (Pyle 1997). Before cognitive testing, all array feeders were maintained in ‘open’ mode, in which feeder doors were open and food was clearly visible and available to all

visiting birds. One week prior to cognitive testing, all feeders were switched to 'all' mode in which all doors were closed but would open when any PIT-tagged bird landed on any feeder perch. This stage allows birds to habituate to the moving doors prior to testing. Following 'all' mode, we started the spatial learning and memory task by programming all feeders to 'target' mode, in which feeders continue to track visits from all birds, but only one feeder on the array opens for each bird with all birds pseudo-randomly assigned to different feeders within an array (Croston et al. 2016, 2017, Tello-Ramos et al. 2018). Following our previous work, we used two metrics to measure cognitive performance in each task: the mean number of 'location errors' (non-rewarding feeders visited prior to visiting the correct rewarding feeder) per trial over the first 20 trials and over the entire task (5 days) (Croston et al. 2016, 2017, Tello-Ramos et al. 2018, Sonnenberg et al. 2019). A trial started when a bird visited any feeder in the array and ended with a visit to the rewarding feeder (Croston et al. 2016). Performance during the first 20 trials of the spatial task represents initial memory acquisition and performance over the entire task reflects longer term memory ability (Sonnenberg et al. 2019). Previously, we have shown that variation in both metrics is associated with fitness consequences (i.e. individuals that make more errors show a fitness reduction) (Branch et al. 2019, Sonnenberg et al. 2019) and has genetic basis (Branch et al. 2022).

Immediately following the spatial learning and memory task, we carried out a single spatial reversal learning task by re-assigning each bird to a different rewarding feeder in each array. Birds assigned to the same feeder during the

spatial learning and memory task were re-assigned to different feeders during the reversal task to prevent social learning (Croston et al. 2017, Tello-Ramos et al. 2018). As in the previous task, we measured performance using the mean number of location errors per trial over the first 20 trials and over the entire spatial reversal learning task (5 days).

We conducted spatial learning and memory tasks from 8 to 13 April in the 2018–2019 winter (both elevations), from 20 to 24 January at low elevation and from 3 to 7 February at high elevation in 2019–2020, and from 13 to 17 January in 2020–2021 (both elevations). We ran spatial reversal learning tasks at both elevations from 13 to 18 April in 2019, from 24 to 29 January at low elevation and from 7 to 13 February at high elevation in 2020, and at both elevations from 20 to 26 January 2021. Variation in testing dates between years and elevations is due to technical difficulties or environmental perturbations (e.g. winter storms, deep snow) that impeded our ability to access the feeders; however, for each test type the data from five consecutive days was used for analyses.

Feather size and growth rate

We collected a single tail feather from juvenile (first year) birds during the autumn and early winter months in 2018–2020 when birds were captured in mist nets prior to the onset of cognitive testing. We removed the single outermost rectrix (r12) from each bird and placed feathers in labelled coin envelopes that were stored in a cool dry place until processing as previous work has shown that feathers do not degrade overtime (Bortolotti et al. 2009). We did not use any

damaged or severely worn feathers in the study. Feathers were assessed for overall mass via an electronic scale to the nearest 0.1 mg and length via ruler to the nearest 0.5 mm. This process was repeated before and after the removal of the calamus or the barbless tip of the feather. Calamus removal was required for the glucocorticoid extraction protocol (see below).

We measured feather growth rate using daily growth bar width (Grubb 2006). Feathers were placed on a white 10.16×15.24 cm notecard and exposed to low-angle light to visualize growth bars. The beginning and end of each growth bar were marked on a notecard in light pencil, allowing for clearer visualization and measurement (Grubb 2006). After marking, each feather card was photographed and ImageJ software (National Institute of Health) was used to measure each growth bar to the nearest 0.1 mm (Schneider et al. 2012). Mean feather growth bar width was used in all analyses.

Feather corticosterone (Cort_f)

We subjected chickadee tail feathers to a standard corticosterone extraction procedure followed by an enzyme-linked immunosorbent (ELISA) assay using methods established by Bortolotti et al. in 2008–2009 and modified following Grant et al. in 2020 (Bortolotti et al. 2008, 2009, Grant et al. 2020). Feathers from all years were extracted in the spring of 2021 and immediately assayed. Individual feathers were cut into multiple pieces (approx. 5 mm in length) and placed (excluding the calamus) into 20 ml scintillation vials with seven mL of HPLC grade 100% methanol. Scintillation vials were sonicated in a

room temperature water bath for 30 min and then placed in a shaking 50°C water bath overnight (16 h). The methanol and feather material were separated using vacuum filtration and each vial was washed twice with two ml of methanol each wash. The separated methanol was then dried in a FlexiVap station, which is composed of a heat block that also supplies constant airflow over the surface of the methanol to aid with evaporation rate. We reconstituted dried samples in 500 ml of assay buffer and samples were sealed and frozen until assayed.

Reconstitution volume and parallelism were determined by previous assays of serial dilutions of chickadee feathers not used in this experiment. We ran samples diluted by 1:300, 1:600 and 1:900. Parallel curves were generated, and a dilution of 1:500 was selected due to this dilution having the highest level of parallelism.

All samples were assayed using a corticosterone ELISA kit supplied by Enzo life sciences (catalog no. ADI-901-097) following the manufacturer's instructions. Feathers from all years were assayed using the same kit to reduce batch bias. We used a serial dilution of known Cort concentration to create a standard curve and used the coefficient of variation (CV) of known standards (20000 pg ml⁻¹, 4000 pg ml⁻¹, 800 pg ml⁻¹, 160 pg ml⁻¹ and 32 pg ml⁻¹) to calculate inter- (18%) and intra-assay (plate 1:15%, plate 2:6%) variation. Inter plate CV was calculated from duplicated controls made from pooled Cort_r from previous samples and was taken through the entire assay process. Cort values were then standardized by feather length to account for variation in Cort_r

deposition during growth (Bortolotti et al. 2008, 2009, Grant et al. 2020). Samples were randomized across plates.

Reproductive monitoring

We predicted yearly variation in $Cort_f$ and feather growth would match the larger population's reproductive pattern (i.e. years with lower resource availability should lead to both higher $Cort_f$ and smaller and lower quality broods). To evaluate annual reproductive output of the study population during the 3 years of study, we analyzed reproductive parameters used in previous studies as an index of environmental and developmental conditions (e.g. (Rodríguez et al. 2016, Kozlovsky et al. 2018)). Mountain chickadees rarely produce more than a single brood each year and all juvenile birds produced in a given season are considered a cohort. Only a handful of nestlings reared in provided nestboxes were recaptured for $Cort_f$ analysis and returned to participate in cognitive testing. However, the individuals that were sampled for both $Cort_f$ and cognitive performance were sampled from the same yearly cohorts, allowing for general comparisons between annual trends in measures of developmental condition and reproductive output. Adult chickadees were monitored throughout the spring and summer months for the onset of breeding behaviour, including nest building in provided nestboxes and first egg date (Kozlovsky et al. 2018). Nests were subsequently monitored on a biweekly basis for onset of incubation, hatching date, brood size and overall success or failure. Nestlings were processed on day 16 post-hatch where we counted brood size, measured individual mass and

banded all nestlings with an aluminium USGS band. Larger clutch and brood sizes and higher fledgling mass is typically associated with better environmental conditions during a given year (Rodríguez et al. 2016). Within-brood CV in fledgling mass was also estimated. Higher CV of fledgling mass indicates a larger variance in condition of individual fledglings within a nest and may reflect relatively worse developmental conditions (i.e. food availability, parental quality, conditions of the nest).

Statistical analyses

All analyses and associated figures for this study were generated using R v. 4.1.2 (Wickham et al. 2019, R Core Team 2021). We used linear and generalized linear models to investigate the relationship between $Cort_f$ and feather morphometrics and individual variation in spatial cognitive performance (mean number of location errors per trial during the first 20 trials and during the entire task) of first-year chickadees. We tested each model for its residual fit using the R package 'DHARMA' (Hartig 2021).

We quantified the presence of corticosterone in each feather in picograms per mm and used these values for the majority of analyses. The only exceptions were analyses that included feather length or mass as a response variable; in these cases, we used the total picogram values per sample. We fit models to test for variation across time and location using year and elevation as fixed effects and $Cort_f$ (controlling for feather mass), feather length (mm), feather mass (g) and mean feather growth rate (mm per 24 h) as response variables. Each

response variable was investigated in an independent model. We ran an additional model with capture time (Julian date) as a fixed effect and $Cort_f$ as the response to test whether earlier sampled individuals had higher $Cort_f$ compared to individuals sampled later in the season. The importance of each fixed effect was tested using a type III Wald chi-square test. When year or elevation was significant, we conducted post hoc Tukey analyses to determine significant differences across levels using the 'emmeans' R package (Lenth 2021).

We fit models testing for $Cort_f$'s relationship with spatial cognitive metrics using $Cort_f$, elevation and year as fixed effects and mean location errors per trial made during the first 20 trials as well as during the entire task (both spatial learning and memory or single spatial reversal learning) as the response variables. For models using mean location errors over the entire trial, we included the total number of trials completed during the task as a control predictor to account for differences in the total number of trials across individuals (Croston et al. 2016, Tello-Ramos et al. 2018). The mean number of errors per trial over the entire task was log-transformed for both the spatial and reversal learning tasks to fit the assumptions of the model. We only used birds that completed a minimum of 20 trials in both tasks for the analyses (Croston et al. 2016, Tello-Ramos et al. 2018). We fit similar models using mean daily feather growth, feather length and feather mass as fixed effects. All initial models were first tested for an interaction between the focal predictor ($Cort_f$, growth, length and mass) and year as well as an interaction between year and elevation. If there was a significant interaction found in the interaction model, each year was

subsequently analyzed separately to investigate the nature of the interaction; otherwise, the interactions were removed. Sample sizes broken down by elevation and year are included in a summary table within the supplementary information.

Lastly, to investigate overall differences among the 3 years of breeding conditions, we fit general linear mixed-effects models using the package 'glmmTMB' (Brooks et al. 2017) to compare reproductive parameters of the nestbox population. We used all available data from each study year despite the vast majority of individuals from the nestbox population not being recaptured for feather analysis or returned to participate in the cognitive tasks. We used elevation and year as fixed effects and breeding pair identification as a random effect to test for differences in clutch size, brood size, mean nestling mass and the CV of nestling mass. Clutch size and brood size models used a generalized Poisson distribution to adjust for count data containing a lack of zero values (Joe & Zhu 2005). Each of these four response variables was run separately but clutch size was also used as a fixed predictor in the brood size model. This allows for the examination of how many nestlings fledged relative to eggs laid.

Results

Variation in feather $Cort_f$, mass, length and growth rate across years and elevations

There were no significant differences in $Cort_f$ between elevations, but $Cort_f$ varied significantly among years (supplementary material, table S1, figure S1A).

In 2018, $Cort_f$ was significantly higher compared to 2019 ($p = 0.04$) and 2020 ($p < 0.001$) and in 2020, $Cort_f$ was significantly lower than in 2019 ($p = 0.015$). Neither variation in feather mass, length, nor 24 h growth rate were significantly associated with differences in $Cort_f$ (supplementary material, table S2); larger, longer and faster growing feathers did not have significantly higher or lower $Cort_f$. Additionally, capture time (Julian date) did not significantly predict $Cort_f$ after controlling for elevation and year showing that feathers collected earlier in the season did not contain more or less $Cort_f$ compared to individuals sampled later (supplementary material, table S2). Capture dates varied from early autumn (20 August) to late winter (29 March), and these results suggest that we were not capturing individuals with more or less $Cort_f$ earlier or later in a given season.

Feather mass did not vary significantly across elevations but there were significant differences across years (supplementary material, table S1, figure S1B). Feather mass was significantly higher in 2019 compared to both 2018 ($p < 0.001$) and 2020 ($p < 0.001$). At the same time, there were no significant differences in feather mass between 2018 and 2020 ($p = 0.44$, supplementary material, figure S1B). Similarly, feathers were significantly longer in 2019 compared to both 2018 ($p = 0.01$) and 2020 ($p = 0.012$), with no significant differences in feather length between 2018 and 2020 ($p = 0.92$) and no differences associated with elevation (supplementary material, table S1). This year-to-year variation was also evident in feather growth rates (i.e. mean feather bar width), which were significantly higher in 2019 compared to 2020 ($p < 0.001$) but not significantly different from growth rate in 2018 ($p = 0.05$). There were no

significant differences in feather growth rate between 2018 and 2020 ($p = 0.23$, supplementary material, table S1, figure S1c), and again there were no significant differences between elevations.

Spatial learning and memory

There was no significant association between performance in the first 20 trials of the learning and memory task (mean number of errors per trial) and $Cort_f$, elevation or year (table 1). However, there was a significant interaction between $Cort_f$ and year (table 1). When analyzed separately within each year, higher levels of $Cort_f$ were associated with worse performance across the first 20 trials (larger mean number of errors per trial) but only in 2018 and only at high elevation (as no data from low elevation were included in this model due to low sample size; table 2; figure 1a). There were no significant associations between performance on the first 20 trials of the task and $Cort_f$ in either 2019 or 2020 (table 2; figure 1b,c).

There were no significant associations between performance in the entire spatial learning and memory task and $Cort_f$, but there was a significant year effect and significant $Cort_f$ by year interaction (table 1). Post hoc analyses showed no statistically significant differences in performance across the entire task between any years (2018|2019: $p = 0.89$; 2018|2020: $p = 0.92$; 2019|2020: $p = 0.99$). There were also no statistically significant associations between the number of location errors per trial over the entire task and $Cort_f$ within each of the 3 years when run in separate independent models (table 1; figure 1d–f).

There were no significant associations between performance either in the first 20 trials of the spatial learning and memory task or during the entire task and mean feather bar width (supplementary material, table S3). Variation in feather length and mass similarly was not associated with differences in cognitive performance (supplementary material, table S3).

Spatial reversal learning

There were no significant associations between $Cort_f$ and performance (mean number of errors per trial) on a single reversal task performance in the first 20 trials (table 1; figure 2a–c) or during the entire task (table 1, figure 2d–f).

There was no significant association between performance in the first 20 trials of the single reversal learning task and mean feather growth bar width, but there was a significant elevation by year interaction (electronic supplementary material, table S3). Post hoc analyses revealed no significant differences between elevations ($p = 0.81$) or between years (2018|2019: $p = 0.18$; 2018|2020: $p = 0.58$; 2019|2020: $p = 0.20$) despite the overall results of the model.

There was no significant association between mean feather bar width and performance over the entire spatial reversal learning task (supplementary material, table S3). Variation in feather mass and length was similarly not associated with differences in performance in a spatial reversal learning task (supplementary material, tables S3 and S4).

Population-level year differences in fledgling condition

Mean fledgling mass was not significantly different between elevations but varied significantly across years and the interaction between elevation and year was statistically significant (supplementary material, table S5). Post hoc analyses confirmed that there were no significant differences between elevations ($p = 0.82$) but there was a significant difference across years with 2020 associated with significantly lower mass compared to 2018 ($p < 0.001$) and 2019 ($p < 0.001$). There were no significant differences in mass between 2018 and 2019 ($p = 0.14$).

The variation of nestling mass within nests (CV) also varied significantly across years but not elevations (supplementary material, table S5). Post hoc analyses showed that the CV of fledgling mass was higher in 2020 compared to both 2018 ($p = 0.005$) and 2019 ($p = 0.012$) but there were no significant differences between 2018 and 2019 ($p = 0.96$).

Discussion

Overall, we found limited support for the hypothesis that the condition during development of wild mountain chickadees results in impaired spatial cognitive abilities. We only detected a significant negative association between spatial cognitive ability and feather $Cort_f$ in the initial memory acquisition phase (first 20 trials) during 1 year of the 3-year study. At the same time, we did not detect a significant association between developmental $Cort_f$ and spatial cognitive ability in the memory retention phase (performance across entire testing period) during any of the 3 years. As the first 20 trials reflect the memory

acquisition phase, while performance over the entire task represents longer term memory ability (Sonnenberg et al. 2019), our results suggest that elevated developmental $Cort_f$ may have limited effects on memory acquisition, but no detectable effects on longer term memory ability. However, as this effect only occurred in a single year this result may be due to chance. We did not detect differences between $Cort_f$ and performance on the single spatial reversal learning task during any year of the study. It is possible that individuals with worse developmental conditions died before our sampling—if this were the case, our results would only be limited to individuals that survived at least a month after fledging and to the age where spatial abilities are relevant to their survival. Although, we did not detect a relationship between $Cort_f$ and sampling date, which argues against the idea that birds with higher $Cort_f$ may die earlier in the post-fledging period. Overall, our results are likely reflective of the relevant naturally existing variation in developmental condition.

Across years, there were significant differences in the total $Cort_f$: mean $Cort_f$ was highest in the 2018 cohort, followed by the 2019 cohort and finally lowest in the 2020 cohort. Despite the detected mean yearly differences, each year was associated with large individual variation. Feather growth, mass and length also varied across years but only 2019 had significantly larger and faster growing feathers compared to both 2018 and 2020. We detected no significant associations between feather growth rates or feather mass and any measure of spatial cognitive performance.

There were no significant differences in cognitive performance across years on either the spatial learning and memory or the spatial reversal tasks. The only exception was a small difference among years in spatial learning and memory performance, but only in models with feather length and mass (see supplemental materials). This disparity is likely to be due to a slight difference in sample size as not all feathers survived the hormone extraction process. These models showed cognitive performance in 2018 (year with the highest $Cort_f$) was worse than 2019 but statistically similar to that in 2020 (the year with the lowest $Cort_f$). Thus, these results corroborate our conclusions that developmental conditions had only a limited effect on spatial cognitive abilities.

There were significant differences in the reproductive parameters among years. Chickadees fledged the lightest massed nestlings in 2020 compared to both 2018 and 2019. The within brood variation in nestling mass was significantly larger in 2020 compared to both 2018 and 2019. All these results suggest that 2020 was associated with lower quality developmental conditions. However, feather $Cort_f$ in 2020 was the lowest among the 3 years of study. These data suggest that there were no broad associations between developmental conditions (as indicated by fledgling body mass and CV of fledgling mass within a nest) and feather $Cort_f$ across years. One explanation for this mismatch is that the pattern seen between years in reproductive output may only be evident at the population level and not the level of the individual. Additionally, there were no striking differences in cognitive performance between years despite large differences in overall developmental conditions.

Despite strong laboratory-based evidence that perturbations during early development can lead to negative impacts on cognitive abilities (e.g. (Hedges & Woon 2011, Buchanan et al. 2013, Naninck et al. 2015, Eyck et al. 2019)), we did not find strong support for this pattern in wild food-caching mountain chickadees tested in their natural environment. This may partly be driven by the inability of laboratory animals to respond to such effects in the absence of resources from natural environments (Buchanan et al. 2013). The basic needs of captive animals are met in the laboratory but if there are compensatory mechanisms mediated via resources only available in natural settings, this may inhibit animals from mounting a compensatory response (Buchanan et al. 2013).

For example, it has been shown that the amino sulfonic acid, taurine can rescue neural tissue and related cognitive functions from the negative effects of environmental stressors (Pasantés-Morales & Hernández-Benítez 2010, Kumari et al. 2013, Jia et al. 2016, Jangra et al. 2020). Taurine is found ubiquitously in nature and while most organisms can synthesize their own source, some avian species may lack this ability during early development and can only gain taurine by consuming certain food sources. For example, spiders contain 40–100 times more taurine than other common arthropod food resources and are a common component of chickadee diets (Ramsay & Houston 2003). Past work has shown that chickadees and close relatives seek out spiders to consume and feed to developing nestlings (Arnold et al. 2007, Van Oers et al. 2015) and that experimental supplementation of taurine results in rescued and in some cases better spatial cognitive abilities (Arnold et al. 2007, Jia et al. 2016, Jangra et al.

2020). Thus, adult chickadees may seek out spider prey to buffer against the negative impacts of developmental stress on structures like the hippocampus, preserving future spatial cognitive function. As a result, additional taurine in the diet may counteract the negative effects of lower daily nutrition due to lower food availability (Pasantés-Morales & Hernández-Benítez 2010, Kumari et al. 2013). Laboratory environments restrict parental behaviors such as selective feeding, as they lack diverse resources, which may explain the consistent negative effects of increased developmental stress on cognitive function (Shettleworth 2009, Buchanan et al. 2013).

Animals in the laboratory are also severely limited in their opportunities to use cognition. This is especially true for spatial cognition—captive environments are well known to result in reduced hippocampal volume (Smulders et al. 1995, LaDage et al. 2009). It is possible that wild animals in their natural environment may be able to counter negative effects of developmental stress on spatial cognitive abilities through extensive use of such abilities later in life (Shettleworth 2009). Food-caching birds cache tens of thousands of individual food caches every autumn and use spatial memory to recover these caches (Pravosudov & Roth 2013). It is possible that this considerable daily use of spatial cognition will compensate or rescue cognitive function from some developmental perturbations (Buchanan et al. 2013).

The use of $Cort_f$ to evaluate nestling condition was established by Bortolotti and colleagues (Bortolotti 2008, 2009). Although, the use of this method for

examining short-term stressors has been discouraged (Romero & Fairhurst 2016), our use of Cort_f as an index of nesting conditions aligns with several other studies that focus on tradeoffs during early development (Will et al. 2014, Johns et al. 2018, Jenni-Eiermann et al. 2022). However, this method does not allow for a temporal understanding of Cort deposition into the feather tissue. A bird with higher Cort_f compared to others in its cohort may have initially experienced significant perturbations that then declined throughout the rest of development. Thus, some individuals with higher Cort_f may have had the ability to recover from this exposure. It is also possible that the detected levels of Cort_f were indicative of system-wide Cort concentrations that were below a harmful threshold. Additionally, there have been some studies showing that poor developmental conditions result in lower levels of glucocorticoid hormones (Heim et al. 2000, Badanes et al. 2011), and that higher levels may be more indicative of a normal function (Patterson et al. 2015). We did see our predicted relationship between higher levels of hormone and worse memory performance but only in a single year and elevation. This result does not remove the possibility that in natural populations higher Cort may be related to more ideal conditions but in either case, variation in developmental conditions in our study is likely representative of naturally present and rather large variation across multiple years in our population.

As the survival of young songbirds during the post-fledging period is very low (Rodríguez et al. 2016), and our study only includes birds that survived at least a few months after fledging, our sample may be biased towards higher-

quality individuals that received lower exposure to developmental harm. On the other hand, laboratory-based studies showed negative effects of rather small differences in developmental stress associated with limited variation in nutrition (Pravosudov et al. 2005, Pravosudov & Kitaysky 2006). If young birds with worse developmental condition indeed die earlier, we would expect samples collected earlier in the autumn to contain higher Cort_r. Yet we detected no such pattern. This suggests that birds with higher levels were not being selected out of the population more quickly during the post-fledging period compared to those with lower levels. Also, if the individuals with worse developmental conditions die soon after fledging, their mortality could not be associated with worse spatial cognitive abilities as these are impossible to sample that early in development in a wild population. This study was completely dependent on individuals that not only survived to fledging but also survived long enough to participate in the cognitive tasks later in the winter.

Overall, our results show that mountain chickadees in their natural environment do not exhibit strong negative effects associated with large natural variation in developmental condition. This may be due to evolved mechanisms allowing them to protect their cognitive abilities during development (Buchanan et al. 2013). However, the nature of such potential mechanisms remains unclear. While future work should focus on untangling such potential mechanisms, this study shows that wild animals in their natural environment may be largely unaffected by naturally existing variation in developmental condition, which is in direct contrast to a large body of laboratory-based research. Our results suggest

that individual cognitive variation in our population is probably not strongly influenced by differences in naturally existing developmental conditions. This emphasizes the importance of investigating basic biological processes in wild animals in their natural environments, as previous results gathered in captive environments may not accurately reflect inherent abilities of these populations.

Ethics

All work was in accordance with the University of Nevada Reno Institutional Animal Care and Use Committee (Protocol 00818, 00046 and 00603), California Department of Fish, and Wildlife Permit D-0011776516-4.

Authors' contributions

B.R.S.: conceptualization, data curation, formal analysis, funding acquisition, resources, writing—original draft, writing—review and editing; V.K.H.: data curation, investigation, writing—review and editing; A.M.P.: data curation, investigation, writing—review and editing; L.M.B.: data curation, investigation, writing—review and editing; C.L.B.: data curation, investigation, writing—review and editing; E.S.B.: funding acquisition, methodology, resources, writing—review and editing; J.Q.O.: conceptualization, methodology, resources, writing—review and editing; V.V.P.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest

We declare we have no competing interests.

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Tables**Table 1** Mean location errors per trial over two spatial tasks and Cort_f

predictor	spatial learning task		single reversal learning task	
	location errors per trial in the first 20 trials <i>n</i> = 101 ^a	location errors per trial in the entire task	location errors per trial in the first 20 trials <i>n</i> = 87 ^b	location errors per trial in the entire task
elevation	$\chi^2_1 = 1.18, p = 0.277$	$\chi^2_1 = 0.13, p = 0.714$	$\chi^2_1 = 0.02, p = 0.874$	$\chi^2_1 = 0.10, p = 0.756$
year	$\chi^2_2 = 2.79, p = 0.248$	$\chi^2_2 = 6.84, p = \mathbf{0.033}$	$\chi^2_2 = 5.82, p = 0.05$	$\chi^2_2 = 4.73, p = 0.094$
Cort _f (pg mm ⁻¹)	$\chi^2_1 = 0.46, p = 0.499$	$\chi^2_1 = 3.77, p = 0.052$	$\chi^2_1 = 0.15, p = 0.702$	$\chi^2_1 = 1.39, p = 0.238$
total trial number	NA	$\chi^2_1 = 81.25, p < \mathbf{0.001}$	NA	$\chi^2_1 = 35.23, p < \mathbf{0.001}$
year × Cort _f	$\chi^2_2 = 8.12, p = \mathbf{0.017}$	$\chi^2_2 = 7.94, p = \mathbf{0.019}$	NA	NA
adjusted <i>R</i> ²	0.06	0.47	0.07	0.38

^aSample sizes by elevation and year for the spatial learning task: 2018 (low: 2; high: 26); 2019 (low: 6; high: 22); 2020 (low: 26; high: 19).

^bSample sizes by elevation and year for the single reversal learning task: 2018 (low: 1; high: 20); 2019 (low: 6; high: 21); 2020 (low: 22; high: 17).

Table 2 Mean location errors per trial over the entire learning and memory task and for the first 20 trials and Cort_t by year.

year	n	predictor	location errors/trial in the first 20 trials	location errors/trial in the entire task
2018	total: 26	elevation	removed due to lack of samples	removed due to lack of samples
	low: 0			
	high: 26	Cort _t (pg mm ⁻¹)	estimate = 0.05 ± 0.02, t = 2.56, p = 0.017	estimate = 0.03 ± 0.01, t = 1.88, p = 0.072
		total trial number	NA	estimate = -2.7 × 10⁻³ ± 5.0 × 10⁻⁴, t = -5.45, p < 0.001
		Adj. R ²	0.21	0.59
2019	total: 28	elevation	estimate = 0.36 ± 0.19, t = 1.93, p = 0.065	estimate = 0.26 ± 0.17, t = 1.54, p = 0.137
	low: 6			
	high: 22	Cort _t (pg mm ⁻¹)	estimate = -4.3 × 10 ⁻³ ± 0.01, t = -0.37, p = 0.712	estimate = -4.6 × 10 ⁻³ ± 0.01, t = -0.46, p = 0.653
		total trial number	NA	estimate = -1.2 × 10⁻³ ± 3.4 × 10⁻⁴, t = -3.64, p = 0.001
		Adj. R ²	0.13	0.46
2020	total: 45	elevation	estimate = 0.05 ± 0.12, t = 0.37, p = 0.708	estimate = -3.0 × 19 ⁻³ ± 0.11, t = -0.03, p = 0.980
	low: 26			
	high: 19	Cort _t (pg mm ⁻¹)	estimate = -6.1 × 10 ⁻³ ± 0.01, t = -0.48, p = 0.635	estimate = -0.01 ± 0.01, t = -0.17, p = 0.169
		total trial number	NA	estimate = -1.4 × 10⁻³ ± 2.4 × 10⁻⁴, t = -5.89, p < 0.001
		Adj. R ²	0.01	0.47

Figure Legends

Fig. 1

Performance on the spatial learning and memory task, showing mean location errors per trial (a–c) during the first 20 trials and (d–f) over the entire task in relation to $Cort_r$ across the 3 years of the study, with high elevation marked in green and low elevation marked in orange.

Fig. 2

Performance on the single reversal spatial learning and memory task, showing mean location errors per trial (a–c) during the first 20 trials and (d–f) over the entire task in relation to $Cort_r$ across the 3 years of the study, with high elevation marked in green and low elevation marked in orange.

Figures

Fig. 1

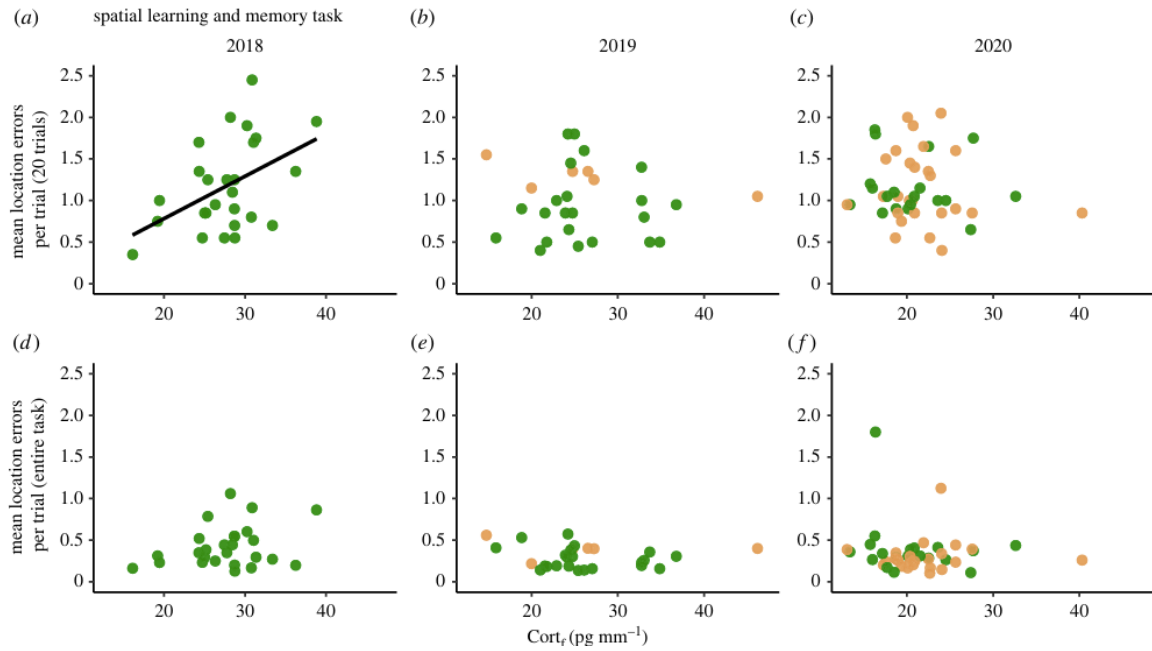
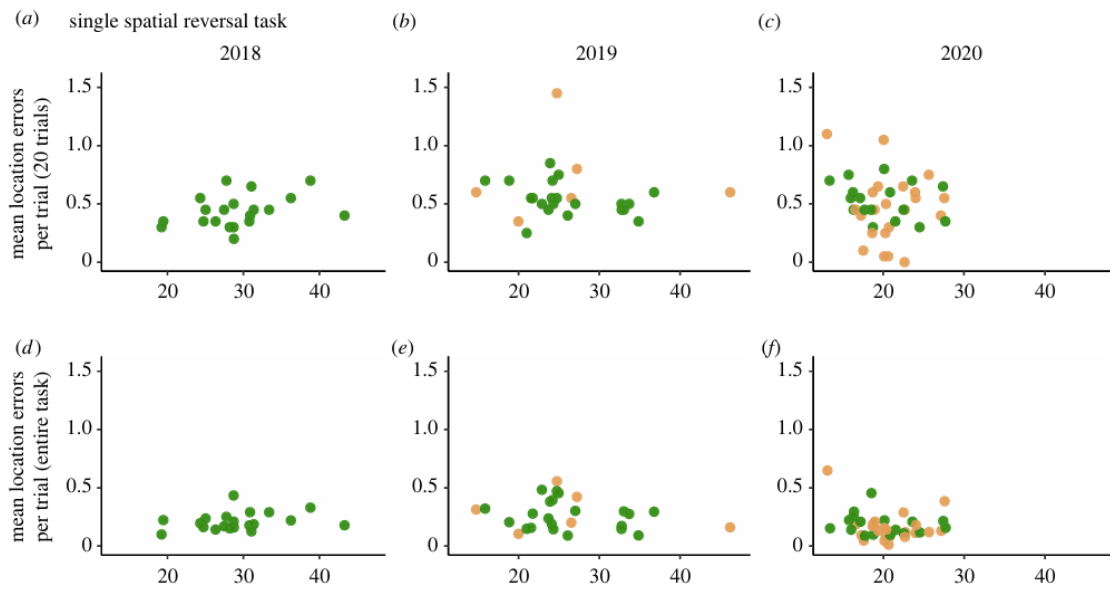


Fig. 2



Supplementary materials

Feather mass, length and growth and spatial cognition

Spatial learning task

There were no significant associations between performance in the first 20 trials of the spatial learning and memory task and feather mass (Table S3). There was a significant association between performance and year and a post hoc analysis showed only a minor difference between 2018 and 2019 with 2019 having slightly better performance associated with smaller mean number of location errors per trial ($p = 0.029$). There were no significant differences between 2018 and 2020 ($p = 0.53$) or 2019 and 2020 ($p = 0.16$).

There were also no significant associations between performance in the entire spatial learning and memory task and feather mass (Table S3). There was a significant effect of year and a post hoc analysis revealed a biologically minor, but significant difference in performance between only 2018 and 2019 ($p = 0.02$) with 2019 having slightly better performance associated with smaller mean number of location errors per trial. There were no significant differences between 2019 and 2020 ($p = 0.09$) or 2018 and 2020 ($p = 0.61$).

There were no significant associations between performance in the first 20 trials of the spatial learning and memory task and feather length (Table 5). There was a significant association between performance and year and a post hoc analysis showed a slight difference in performance but only between 2018 and 2019 ($p = 0.016$) with 2019 having better performance. There were no significant

differences in performance between 2019 and 2020 ($p = 0.18$) or 2018 and 2020 ($p = 0.42$).

There were no significant associations between performance during the entire task of the spatial learning and memory task and feather length (Table 5). There was a significant year effect, and a post hoc analysis showed the same previous trend wherein there was a slightly better performance in 2019 compared to 2018 ($p = 0.046$). There were no significant differences in performance between 2019 and 2020 ($p = 0.15$) or 2018 and 2020 ($p = 0.69$).

Single reversal spatial task

There was no significant association between performance in the first 20 trials of the single spatial reversal learning task and feather mass (Table S3), but there was a significant elevation by year interaction (Table S3). However, post hoc analyses revealed no significant differences between elevations ($p = 0.77$) or year (2018 | 2019: $p = 0.22$; 2018 | 2020: $p = 0.58$; 2019 | 2020: $p = 0.29$). There was no significant association between feather mass and performance over the entire reversal task (Table S3).

There was a significant association between feather length and performance in the first 20 trials of the single spatial reversal learning task as well as a significant year by elevation interaction (Table S3). We analyzed each year separately and found that there was no significant association between performance and feather length in 2018 (Table S4). However, this model only involved high elevation as low elevation was removed due to low sample size.

There was no significant association between performance and feather length in 2019 but there was a significant elevation effect (Table S4). In 2020, there was a significant association between performance during the first 20 trials and feather length (Table S4); individuals with longer feathers performed worse by making more location errors per trial. This result may potentially be due to chance as it only occurred in a single year, it was in the opposite direction from expectation, as larger feathers sometimes point to better development and thus would be expected to predict better performance on the cognitive task (59).

There was no significant association between feather length and performance across the entire reversal task (Table S3).

Population level year differences in reproductive parameters.

There were significant differences in clutch size between elevations and across years, with a significant elevation by year interaction (Table S5). Post hoc analyses revealed significant differences between elevations in clutch size with low elevations having larger clutches but only in 2020 ($p < 0.001$). There were also significant differences in mean clutch size between years with 2018 having significantly larger clutches compared to 2019 ($p = 0.007$).

Brood sizes showed no significant differences between elevations but were significantly different across years while controlling for clutch size (Table S5, Figure S1). Post hoc analyses showed that 2020 was associated with significantly smaller brood sizes compared to both 2018 ($p = 0.032$) and 2019 (p

= 0.037). There was no significant difference in brood size between 2018 and 2019 ($p = 1.00$).

Supplemental Tables

Table S1

Feather characteristics and reproductive parameters of the same years				
Response	N	Predictor		Adjusted R ²
Cort _f (pg/mm)	134	Elevation	$\chi^2_1 = 0.29, p = 0.587$	0.20
		Year	$\chi^2_2 = 28.66, p < 0.001$	
Feather mass (mg)	157	Elevation	$\chi^2_1 = 1.34, p = 0.247$	0.15
		Year	$\chi^2_2 = 29.37, p < 0.001$	
Feather length (mm)	153	Elevation	$\chi^2_1 = 0.45, p = 0.503$	0.05
		Year	$\chi^2_2 = 11.40, p = 0.003$	
Feather growth rate (mm/24hr)	147	Elevation	$\chi^2_1 = 2.44, p = 0.118$	0.11
		Year	$\chi^2_2 = 19.41, p < 0.001$	

Table S2

Feather size, Cort _f and time of capture				
Response	N	Predictor		Adj R ²
Mass (mg)	134	Cort _f (pg)	$\chi^2_1 = 2.76, p = 0.96$	0.14
		Elevation	$\chi^2_1 = 3.28, p = 0.070$	
		Year	$\chi^2_2 = 19.44, p < 0.001$	
Length (mm)	134	Cort _f (pg)	$\chi^2_1 = 0.10, p = 0.752$	0.08
		Elevation	$\chi^2_1 = 0.40, p = 0.524$	
		Year	$\chi^2_2 = 14.39, p < 0.001$	
Feather growth rate (mm/24hr)	129	Cort _f (pg)	$\chi^2_1 = 0.08, p = 0.773$	0.08
		Elevation	$\chi^2_1 = 2.16, p = 0.142$	
		Year	$\chi^2_2 = 12.61, p = 0.002$	
		Feather mass (mg)	$\chi^2_1 = 0.10, p = 0.841$	
		Feather length (mm)	$\chi^2_1 = 2.03, p = 0.154$	
Cort _f	134	Capture time (Julian date)	$\chi^2_1 = 0.08, p = 0.783$	0.19
		Elevation	$\chi^2_1 = 0.25, p = 0.618$	
		Year	$\chi^2_2 = 23.78, p < 0.001$	

Table S3

Mean location errors per trial over two spatial tasks and feather length, mass and mean feather bar width				
Predictor	Spatial learning task		Reversal learning task	
	Location errors/ trial in the first 20 trials N = 119	Location errors/ trial in the entire task	Location errors/ trial in the first 20 trials N = 101	Location errors/ trial in the entire task
Elevation	$\chi^2_1 = 0.87, p = 0.350$	$\chi^2_1 = 0.02, p = 0.884$	$\chi^2_1 = 0.17, p = 0.683$	$\chi^2_1 = 0.06, p = 0.800$
Year	$\chi^2_2 = 7.06, p = 0.029$	$\chi^2_2 = 8.07, p = 0.018$	$\chi^2_2 = 2.60, p = 0.273$	$\chi^2_2 = 1.62, p = 0.444$
Feather mass (mg)	$\chi^2_1 = 1.14, p = 0.286$	$\chi^2_1 = 3.81, p = 0.051$	$\chi^2_1 = 2.96, p = 0.085$	$\chi^2_1 = 0.71, p = 0.398$
Total trial number	NA	$\chi^2_1 = 85.64, p < 0.001$	NA	$\chi^2_1 = 51.40, p < 0.001$
Elevation * Year	NA	NA	$\chi^2_2 = 9.04, p = 0.011$	NA
R ²	0.03	0.45	0.13	0.40
N = 115				
Elevation	$\chi^2_1 = 1.21, p = 0.271$	$\chi^2_1 = 0.004, p = 0.947$	$\chi^2_1 = 0.14, p = 0.710$	$\chi^2_1 = 0.07, p = 0.796$
Year	$\chi^2_2 = 8.16, p = 0.017$	$\chi^2_2 = 6.50, p = 0.039$	$\chi^2_2 = 2.04, p = 0.360$	$\chi^2_2 = 2.19, p = 0.335$
Feather length (mm)	$\chi^2_1 = 2.76, p = 0.097$	$\chi^2_1 = 2.84, p = 0.092$	$\chi^2_1 = 7.92, p = 0.005$	$\chi^2_1 = 1.00, p = 0.318$
Total trial number	NA	$\chi^2_1 = 78.35, p < 0.001$	NA	$\chi^2_1 = 46.70, p < 0.001$
Elevation * Year	NA	NA	$\chi^2_2 = 8.68, p = 0.013$	NA
R ²	0.12	0.48	0.17	0.40
N = 109				
Elevation	$\chi^2_1 = 0.72, p = 0.395$	$\chi^2_1 = 0.10, p = 0.753$	$\chi^2_1 = 0.05, p = 0.830$	$\chi^2_1 = 0.10, p = 0.748$
Year	$\chi^2_2 = 2.74, p = 0.254$	$\chi^2_2 = 3.74, p = 0.154$	$\chi^2_2 = 4.08, p = 0.130$	$\chi^2_2 = 2.95, p = 0.229$
Mean feather bar width (mm/24hr)	$\chi^2_1 = 0.11, p = 0.743$	$\chi^2_1 = 0.62, p = 0.429$	$\chi^2_1 = 1.26, p = 0.261$	$\chi^2_1 = 0.23, p = 0.632$
Total trial number	NA	$\chi^2_1 = 76.89, p < 0.001$	NA	$\chi^2_1 = 40.65, p < 0.001$
Elevation * Year	NA	NA	$\chi^2_2 = 7.05, p = 0.029$	NA
R ²	0.13	0.48	0.20	0.41
N = 95				

Table S4

Mean location errors/trial for the first 20 trials in the reversal task and Cort-			
		Errors in the first 20 trials	
Year	N	Predictor	
2018	Total: 23 Low: 0 High: 23	Elevation Feather length (mm) R ²	Removed due to lack of samples estimate = 0.01 ± 0.01 , $t = 1.03$, $p = 0.317$ 0.002
2019	Total: 34 Low: 11 High = 23	Elevation Feather length (mm) R ²	estimate = 0.23 ± 0.08, $t = 2.66$, $p = 0.012$ estimate = 0.02 ± 0.02 , $t = 1.33$, $p = 0.193$ 0.17
2020	Total: 42 Low: 22 High: 20	Elevation Feather length (mm) R ²	estimate = -0.05 ± 0.07 , $t = -0.69$, $p = 0.493$ estimate = 0.04 ± 0.02, $t = 2.35$, $p = 0.024$ 0.10

Table S5

Reproductive parameters across 2018 - 2020				
Response	N	Predictor		R ²
Clutch size (egg number)	338*	Elevation	$\chi^2_1 = 3.89, p < 0.048$	0.48
		Year	$\chi^2_2 = 10.30, p = 0.006$	
		Elevation * Year	$\chi^2_2 = 12.67, p = 0.002$	
Brood size (fledgling number)	282**	Elevation	$\chi^2_1 = 0.11, p = 0.742$	0.45
		Year	$\chi^2_2 = 8.36, p = 0.015$	
		Clutch	$\chi^2_1 = 192.27, p < 0.001$	
Mean nestling mass (g)	280***	Elevation	$\chi^2_1 = 1.13, p = 0.287$	0.62
		Year	$\chi^2_2 = 12.34, p = 0.002$	
		Elevation * Year	$\chi^2_2 = 6.17, p = 0.046$	
Coefficient of variation of nestling mass (g)	280***	Elevation	$\chi^2_1 = 0.26, p = 0.607$	0.17
		Year	$\chi^2_2 = 12.11, p = 0.002$	

*Sample sizes by elevation and year (2018: low: 67; high: 45; 2019: low: 70; high: 38; 2020: low: 64; high: 54)

**Sample sizes by elevation and year (2018: low: 53; high: 40; 2019: low: 65; high: 30; 2020: low: 53; high: 41)

*** Sample sizes by elevation and year (2018: low: 53; high: 40; 2019: low: 65; high: 30; 2020: low: 53; high: 39)

Table S6

Samples sizes broken down by elevation, year and analysis					
	Total	Elevation	2018	2019	2020
Cort _r ~ elevation + year: table S1	N = 134	Low	4	11	33
		High	33	24	29
Feather mass ~ elevation + year: table S1	N = 157	Low	8	17	34
		High	36	30	32
Feather length ~ elevation + year: table S1	N = 153	Low	7	17	34
		High	35	28	32
Feather bar width ~ elevation + year: table S1	N = 147	Low	7	17	34
		High	32	25	32
Feather mass ~ Cort + elevation + year: table S2	N = 134	Low	4	11	33
		High	33	24	29
Feather length ~ Cort + elevation + year: table S2	N = 134	Low	4	11	33
		High	33	24	29
Feather bar width ~ Cort + elevation + year: table S2	N = 129	Low	4	11	33
		High	30	22	29
Cort ~ Julian date + elevation + year: table S2	N = 134	Low	4	11	33
		High	33	24	29
Location errors (spatial learning) ~ feather mass + elevation + year: table S3	N = 119	Low	3	12	26
		High	29	27	22
Location errors (reversal learning) ~ feather mass + elevation + year: table S3	N = 101	Low	1	11	22
		High	23	24	20
Location errors (spatial learning) ~ feather length + elevation + year: table S3	N = 115	Low	2	12	26
		High	28	25	22
Location errors (reversal learning) ~ feather length + elevation + year: table S3	N = 99	Low	1	11	22
		High	22	23	20
Location errors (spatial learning) ~ feather bar width + elevation + year: table S3	N = 109	Low	2	12	26
		High	25	22	22
Location errors (reversal learning) ~ feather bar width + elevation + year: table S3	N = 95	Low	1	11	22
		High	20	21	20

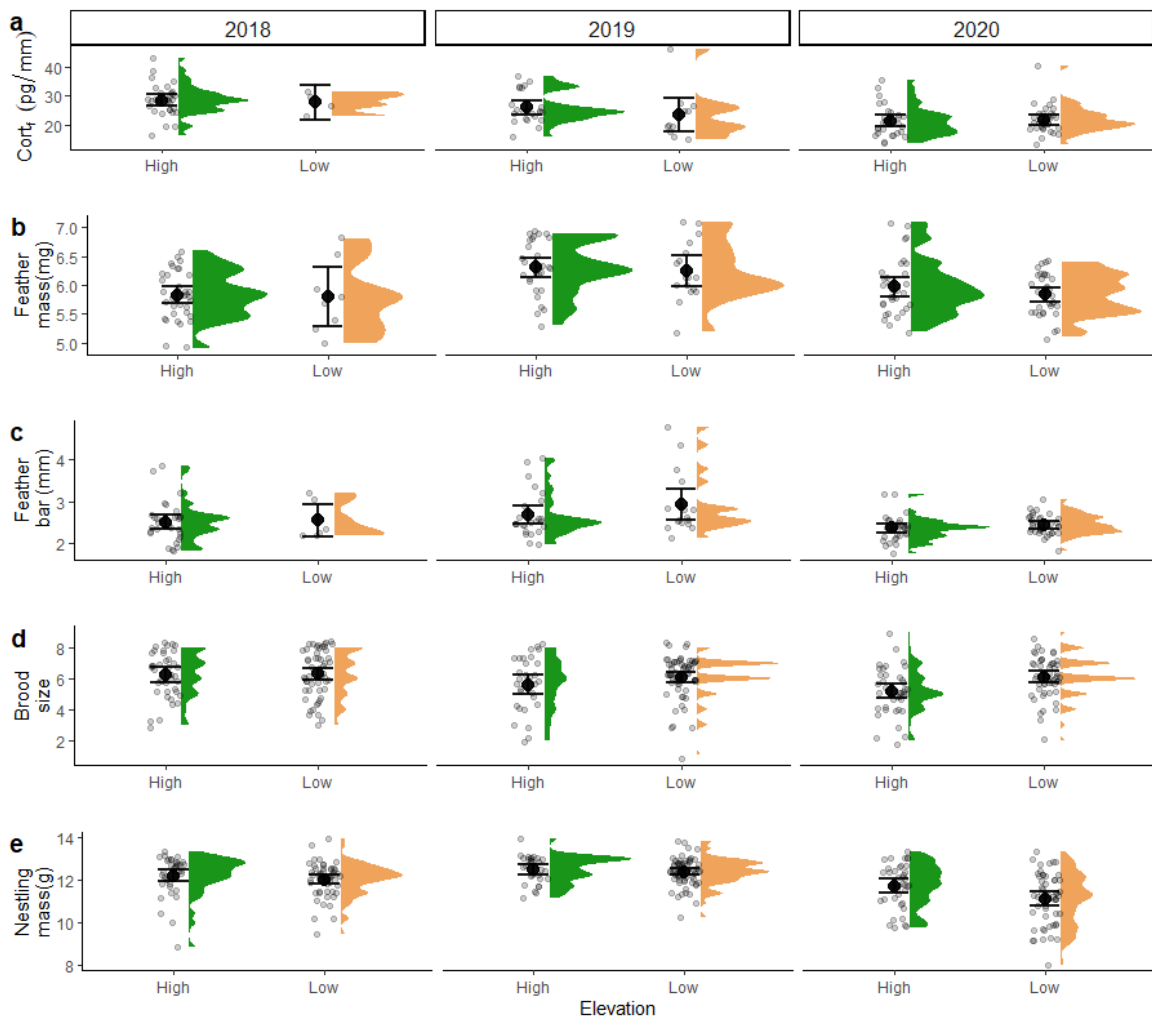
Supplementary Fig. Legends

Fig. S1

The mean comparison across years and elevations for a) $Cort_f$ per millimeter of feather, b) feather mass, c) mean feather bar width d) brood sizes for the same years of the study and d) mean nestling mass for the same years of the study

Supplementary Fig.

Fig. S1



Conclusion

Overall, understanding the mechanisms that drive complex behavioral trait variation in wild systems remains a challenging goal within the field of behavioral ecology (Trillmich et al. 2015). However, my dissertation work provides some of the first evidence showing that variation in these traits is being limited by directional natural selection. Additionally, I showed limited evidence for multiple factors of the developmental environment acting on offspring fitness.

My first chapter showed evidence for directional selection acting on the spatial cognitive abilities involved in the recovery of thousands of individual food stores in food-caching mountain chickadees in harsh high elevation environments. This study provided strong evidence supporting a long-standing hypothesis that harsh environments may drive better spatial memory abilities needed to recover food caches to survive the winter (Pravosudov & Clayton 2002, Pravosudov & Roth 2013). These abilities are reliant upon specialized neural architecture (i.e., the hippocampus) that is energetically expensive to develop and maintain (Smulders et al. 2015, Freas et al. 2013, Pravosudov & Roth 2013). My dissertation adds to the knowledge of how differences in development environment may shape the variation in these costly traits.

I examined multiple aspects of the developmental environment of chickadees. This included measuring the variation in the local climate (including the microclimate and ambient temperatures) across each discrete developmental period. I also collected and dissected nests to examine their composition,

ectoparasite load, and the immune responses of the offspring within each nest. Despite considerable variation, I found no discernible fitness consequences for the offspring in response to these developmental environments.

However, I did find an interesting pattern of within-individual repeatable behavior (Bell et al. 2009, Trillmich et al. 2015). First, female chickadees build similar sized nests and nests of similar composition across years. This was the case across a drought year (very little snow and warmer temperatures) and a year with above average snow levels (providing cooler temperatures to offspring). This evidence shows that nest building behavior may be heritable and that variation in nest structure is explained by individuals rather than by environmental conditions (van Oers et al. 2004, Bell et al. 2009). Interestingly, this nest building behavior was shown to be highly correlated with food-caching propensity, which is also highly repeatable within individuals, despite these behaviors being functionally and seasonally independent (McCallum et al. 2020). These findings show that a highly functional behavior under strong selection (i.e., food-caching) can influence a behavior with very little consequences to fitness (i.e., nest building) and thus account for some of the observed variation in the latter trait. Indeed, this indicates a potential behavioral syndrome with some shared mechanism that may underly both of these behaviors (Sih et al. 2004a,b).

Lastly, I used feathers grown in the nest to evaluate developmental conditions and tested for a relationship between developmental burden and cognitive performance in chickadees in their first year of life. There were no

signification associations between any feather growth measurement and cognitive performance, which suggests that chickadees may have evolved some compensatory buffering mechanisms that protect specialized spatial cognitive abilities critical for survival from developmental challenges.

Overall, my dissertation provides novel data on causes and consequences of trait variation using two important behavioral traits, spatial cognition and nest building behavior, and identifies directions for future studies, specifically work targeting the role of the developmental environment on the formation of spatial cognitive abilities in wild populations.

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