University of Nevada, Reno

The relationship between environment, behavior, cognition, and the brain, in specialized food-caching chickadees (*Poecile gambeli*)

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

By

Dovid Y. Kozlovsky
Dr. Vladimir Pravosudov/Advisor

December, 2017

THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

DOVID KOZLOVSKY

Entitled

The Relationship Between Environment, Behavior, Cognition, And The Brain, In Specialized Food-Caching Chickadees (Poecile Gambeli)

be accepted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

Vladimir Pravosudov, PhD, Advisor

Annie Leonard, PhD, Committee Member

Chris Feldman, PhD, Committee Member

Matt Forister, PhD, Committee Member

Alexander van der Linden, PhD, Graduate School Representative

David W. Zeh, Ph. D., Dean, Graduate School

December, 2017

Abstract

Environmental heterogeneity is known to affect phenotypic variation. Behavioral traits and the brain regions controlling these traits may be especially variable across environmental gradients as behavioral traits change rapidly in response to environment. Behavioral traits have been shown to differ across several environmental gradients of climatic harshness and novelty, such as latitudinal, elevational and urbanization gradients. This dissertation focuses on how cognition, behavior and the brain differ food-caching specialists inhabiting environments that differ in climatic harshness (i.e. differ in elevation) and novelty (i.e. differ in anthropogenic activity). I found that, chickadees from harsher high elevations, when compared with low elevation chickadees, have better problem-solving abilities and that these chickadees with better cognition are less willing to take risks when perceived predation risk is high, which resulted in a reduced investment in current offspring. I also found that chickadees from urban environments had a suite of generalist traits (e.g. more active in exploring a novel environment, better problem-solving abilities and larger brains) and some food-caching specialist traits (e.g. better long-term spatial memory retention) when compared with forest chickadees. This dissertation highlights that unique suites of behavioral traits are associated with different environments and suggests that a better understanding of how specific environmental factors affect specific (suites of) traits is necessary.

Background

The environment an organism inhabits is generally assumed to affect its phenotype (e.g. Endler 1986; Freas et al. 2012; Grant & Grant 2002; Roth et al. 2010; Roth & Pravosudov 2009; Via & Lande 1987). Environment-associated changes in phenotype can occur over multiple generations via natural selection or within an individual's lifetime via phenotypic plasticity (e.g. Chevin et al. 2013; Miranda et al. 2013; Sol et al. 2013). Regardless of the mechanism, populations of a given species throughout their geographic range may contend with a number of different environments, and therefore may be expected to differ in a suite of phenotypic traits associated with those particular environments.

Three environmental gradients in particular have received a tremendous amount of attention in the literature: 1) latitudinal, 2) elevational, and 3) anthropogenic disturbance gradients. Along all three of these gradients, differences in abiotic and biotic factors have been shown to alter a wide range of morphological and behavioral phenotypes(e.g. latitude: Griebeler et al. 2010; Riechert & Jones 2008; Robberecht et al. 1980; Roth et al. 2010; Roth & Pravosudov 2009; Vandewoestijne & Van Dyck 2011; elevation: Badyeav & Ghalambor 2001; Cordell et al. 1998; Emery et al. 1994; Freas et al. 2013; Freas et al. 2012; Johnson et al. 2006; urbanization: Evans et al. 2011; Kark et al. 2007; Maklakov et al. 2011; Mendes et al. 2011; Partecke & Gwinner 2007; Sih et al. 2011; Sol et al. 2013; Zhang et al. 2011). Differences in behavioral phenotypes, which are under control by the brain, may be especially important for how many animals cope with their environments. Behavior can allow animals to rapidly respond to their environment, and behavior can be rapidly altered by environmental changes (e.g. Carlton 1963; Cheil & Beer 1997). In light of the instrumental role of behavior in mediating interactions between animals and the environment in which they dwell, my dissertation focuses on behavioral traits; specifically, those that pertain to cognition and the brain. Cognitive traits, defined as traits that allow animals to process, code, recall information and then behave based on this information (Shettleworth 1998), are a suite of behavioral traits thought to be especially important for animals in unpredictable, harsh, and novel environments (Sol et al. 2005; Roth & Pravosudov 2009; Roth et al. 2010). These cognitive traits are often thought to be associated with increased foraging success (e.g. Cole et al. 2012), but may also be costly to produce and maintain (Cole et al. 2013; Coppens et al. 2010; Dukas 1999; Healy 2012; Kawecki 2010; Kotrschal et al. 2013; Kozlovsky et al. 2014a; Morand-Ferron et al. 2016; Niemela et al. 2013). As such, variation in cognition has strong implications for animals contending with different environmental conditions.

Both latitudinal and elevational gradients are associated with differences in climatic severity, and thus climate-driven differences in behavioral responses (and the brain regions underlying these behaviors) may be expected (e.g. Croston et al. 2015; Freas et al. 2013; Freas et al. 2012; Kozlovsky et al. 2014a, b; Roth et al. 2010; Roth & Pravosudov 2009). Latitudinal gradients exist over large spatial scales and therefore, climate may not be the only variable that differs, while environmental differences across elevational gradients usually occur rapidly over extremely small spatial scales and may, therefore, present a more interesting and straightforward framework in which to investigate the relationship between the environment and phenotypic variation. Additionally, elevation gradients present the opportunity for logistically feasible replication both on different peaks within a mountain range and across different mountain ranges, and this replication can be carried out within species or among closely related species with similar and different ecological niches. Our laboratory has previously focused on both latitudinal and elevation gradients, but this dissertation will focus specifically on the small spatial elevation gradient found along mountain slopes in the Sierra Nevada Mountains.

Given the multitude of potentially competing biotic and abiotic factors that can drive differences in phenotype, it is often hard to tease apart the specific factors responsible for phenotypic differences (Endler 1986; Merila & Hendry 2014; Morand-Ferron et al. 2016), and whether these phenotypic differences arise from selection on a particular trait (or a suite of traits) or from phenotypic plasticity (Croston et al. 2015; Lowry et al. 2013; Morand-Ferron et al. 2016), or both. For example, in our study system of food-caching Parids along a latitudinal gradient, high latitude black-capped chickadees (*Poecile atricapillus*) that contend with harsher winter environments have been shown to cache more food, to have better spatial memory acquisition and retention associated with larger hippocampi, and a larger total number of hippocampal neurons and increased adult hippocampal neurogenesis rates compared to lower latitude chickadees (Roth et al. 2010; Roth & Pravosudov 2009). While all indirect evidence suggests that these phenotypic differences are a result of natural selection, the methods employed could not rule out maternal effects or early developmental experiences (Croston et al. 2015). Regardless of these limitations, understanding, generally, how variation in environmental conditions can affect behavior and the associated brain structures and processes can provide clues to how behavior and the brain will respond to changing environments. Because behavior is highly sensitive to environmental conditions and can rapidly change in response to the environment (e.g. Atwell et al. 2014), behavior may be a useful trait for the early detection of rapid environmental changes, such as those that may occur with human-induced change. Given that Black-capped chickadees along a latitudinal gradient of climatic severity differed in novel problem-solving and responses to novel stimuli, my first chapter focuses on how a closely-related species, mountain chickadees (*Poecile gambeli*), differ in these two cognitive traits at a short spatial scale in montane environments that differ in climatic severity.

However, attributes of cognition and the brain are not the only traits expected to vary across environmental gradients. Differences in other phenotypes, including reproductive investment, are also predicted to change in response to unpredictable or challenging environmental conditions (e.g. Boretto et al. 2014; Giesel 1976; Marshall et al. 2008; Martin & Wiebe 2004; Holberton & Wingfield 2003). These differences in reproduction in, differences in environments have been widely assumed to affect the dynamics of life history (Badyaev & Ghalambor 2001; Balasubramaniam & Rotenberry 2016; Bears et al. 2009; Boyle et al. 2016; Partridge & Harvey 1988; Southwood 1988; Williams 1966). Differences in environmental factors can affect both survival across different age classes and the ability of individuals to invest in growth and reproduction (see Badyaev & Ghalambor 2001). Life history theory predicts that longer-lived species should be less fecund and should invest less in current offspring and more in their own survival (Ghalambor & Martin 2000; Trivers 1974; Williams 1966). The reason is that longer-lived species have the potential for many future reproductive events and therefore, can maximize their lifetime reproductive output by reducing risks associated with any particular reproductive event via allocating current energy for maintenance and growth, allowing for successful future reproduction (Badyaev & Ghalambor 2001; Curio 1988; Ghalambir & Martin 2000, 2001; Ghalambor et al. 2013; Trivers 1974). Failing one reproductive event should still allow for future reproduction over multiple years, which should lead to greater fitness as compared to a single reproduction season. Short-lived species, on the other hand, are expected to maximize fitness by allocating all available resources to current offspring, potentially at the expense of their own life, as the chances of producing future offspring are lower. A classic example of this is seen in guppies, where differential predation risk in different environments led to differences in associated life history strategies (Endler 1995). Another example based on phylogenetically-paired species inhabiting different elevations suggested that high elevation parents produce fewer offspring, but invest more heavily in each individual offspring resulting in increased juvenile survival (Badyaev & Ghalambor 2001).Variation in the predictions of life history theory do occur both among and within species when accounting for the multitude of factors that can affect survival and/or reproduction (e.g. Bears et al. 2009; Boyle et al. 2016; Charnov 1991; Crespi et al. 2013; Dunkel et al. 2014; Liao et al. 2016), and in some cases, variation in cognition may explain these deviations. For example, it has been suggested that animals with enhanced cognitive traits have increased survival while animals with worse cognition have decreased survival (Pravosudov & Roth 2013; Sol et al. 2005, 2007), and cognition is not often considered when addressing differences in life history. If differences in cognition are associated with differences in survival, then animals with enhanced cognition may live longer and animals with worse cognition may be shorter-lived, providing one potential explanation to deviations from the predictions of life history theory. In chapter 2, I use this foundation of life history theory to test for potential differences in parental risk taking in mountain chickadees that differ in cognitive traits living at two elevations, and contrast two hypotheses driven by the logic of life history theory to an alternative hypothesis relating to cognition and parental risk-taking (Cole et al. 2012; Healy 2012).

In addition to naturally occurring environmental gradients, rapidly expanding urbanization presents unique challenges but also opportunities for those animals that can invade and exploit these novel environments (Kark et al. 2007; Lowry et al. 2013; Moller 2009). Urban environments are characterized by novel stimuli, altered landscapes, novel biotic interactions, and novel resources (Kark et al. 2007; Lowry et al. 2013). Animals that invade these novel urban environments must face unique features of these anthropogenic centers that can drive phenotypic changes in behavior and the brain resulting in successful invasion and establishment (e.g. Lowry et al. 2013; Maklakov et al. 2011; Miranda et al. 2013; Sol et al. 2013). Generalist species which have a broader environmental tolerance and can utilize a variety of resources are often thought to be more successful in urban environments compared to more specialist species which have a narrower environmental tolerance and specialize on relatively few resources (Bateman & Fleming 2012; Bonier et al. 2007; Chace & Walsh 2006; Evans et al. 2012; Lowry et al. 2013; Marzluff 2017). This hypothesis is, in part, driven by the fact that specialists are expected to do best in environments that are relatively uniform in space and time compared to generalists (Devictor et al. 2008) and urban environments tend to be more heterogeneous both in space and time and are often associated with a reduction in native vegetation and an abundance of novel resources. While specialists might be expected to be less successful in invading and adjusting to novel and urban environments, in these species behaviors related to their specialization may also be important contributors to their success in human-altered landscapes. Because urbanization is an ever-increasing phenomenon, it is important to understand how animals, especially those that are highly specialized (either by having a narrow ecological niche or a narrow environmental tolerance), might adjust to urban dwelling. At the same time, the novelty of the urban environments makes it hard to identify which behaviors might predispose some animals to be successful in the city and what effects city dwelling might have on phenotypes of urban animals. Chapter 3 of my dissertation seeks to understand what cognitive, behavioral, and neural traits are associated with the success of a specialized species in an urban center.

My dissertation aims to understand how different environments might affect animals in general, and food-caching specialist in particular by using a small, specialized, food-storing species, mountain chickadees (*Poecile gambeli*), as a study system. Mountain chickadees are an abundant species in the Sierra Nevada Mountains and the nearby city of Reno, NV. Mountain chickadees are non-migratory resident passerines inhabiting the montane regions of Western North America that specialize on pine seeds, and store thousands of seeds during fall to survive the winter (Mccallum et al. 1999). Winter survival requires remembering numerous locations of these stores, which, in turn, relies on spatial memory and the hippocampus (the brain region involved in spatial memory; Sherry & Vaccarino 1989; Krebs et al. 1989; Pravosudov & Roth 2013). During winter, chickadees are highly social, forming stable winter flocks of unrelated individuals characterized by linear dominance hierarchy (Ekman 1989; Mccallum et al. 1999). Flocks are formed post-breeding and are maintained until the following breeding season (Ekman

1989; Mccallum et al. 1999). During the winter, pairs within the flock are formed so that when the breeding season begins anew, mates have already been chosen (Ekman 1989). During the breeding season, chickadees switch food resources to a diet consisting mainly of invertebrates, which are also the main resource fed to young (Mccallum et al. 1999). Mountain chickadees are quite common in the Sierra Nevada Mountains and my dissertation research takes advantage of our laboratory's well-established field site along an elevation gradient in Tahoe National Forest, well-established protocols for behavioral and brain research in the laboratory, and the (relatively) new population of chickadees in Reno to address these research goals.

The purpose of this dissertation is to investigate cognitive and behavioral traits associated with animals inhabiting different environments and how these traits might impact aspects of parental investment. This dissertation focuses on food-caching animals as a study system, because of the strong theoretical foundation that links food-caching, certain aspects of cognition (e.g. spatial memory and the hippocampus), the environment, and fitness. The results, however, in many instances may be broadly applicable to other animals that utilize cognitive and behavioral traits differentially depending on the environmental context.

Ch. 1: Problem solving ability and response to novelty in mountain chickadees (*Poecile gambeli*) from different elevations

Dovid Y. Kozlovsky, Carrie L. Branch, and Vladimir V. Pravosudov

Abstract

Animals inhabiting challenging and harsh environments are expected to benefit from certain phenotypic traits including cognitive abilities. In particular, innovation and habituation are traits thought to benefit animals in challenging environments and increase individual's probability of survival via increased foraging success. Here we tested whether mountain chickadees from two elevations varying in winter climate severity differ in two traits involving innovation and habituation - problem-solving ability and response to novelty. Higher montane elevations are associated with a significantly longer winter period characterized by lower temperatures and more snow making winter survival more challenging due to a probable increase in foraging demands. Mountain chickadees inhabiting the harsher high elevation were significantly faster at solving a novel problem than their low elevation counterparts. Birds from both elevations responded with increased latency to approach the novel object, however, there were no elevation related differences. Male and female chickadees responded similarly on both tasks, suggesting no sex related differences in problem solving ability or neophobia. The problem solving results are consistent with the results for closely related black-capped chickadees along a longitudinal gradient of winter climatic harshness on a larger geographic scale, but the response to novelty data are not. Overall, our data supports the

hypothesis that enhanced problem solving ability might be associated with living in harsher environments either via natural selection or by plastic response to different environments and suggest that differences in problem solving ability do not have to be associated with differences in neophobia.

Introduction

Animals living in challenging (e.g. harsh and/or unpredictable) or novel environments (an environment to which an animal has had no prior exposure) are thought to benefit from having certain behavioral traits, which allow for increased foraging success (Sol et al. 2005; Roth et al. 2009, 2010). Population and/or individual variation in these phenotypic traits may arise through natural selection or through phenotypic plasticity due to different experiences/environments. One group of traits that is thought to be favored in challenging and novel environments is increased cognitive abilities (Dukas 1998; Shettleworth 1998, 2009; Roth et al. 2010; Wright et al. 2010). Enhanced cognitive abilities are suggested to be associated with increased survival via increased foraging success (Cole et al. 2012) and might be especially important in harsh environments where energetic demands might be higher (because of lower temperatures) and where food may be severely limited during winter season (Roth et al. 2010).

Two of the most extensively studied traits that may be particularly important in challenging or novel environments are problem solving ability and response to novelty. While problem solving ability has been widely regarded as a cognitive trait (e.g. Roth et al. 2010; Cole et al. 2012) -- based on the definition of cognitive traits as traits that aid in information processing (Rowe and Healy 2014) -- there is a debate about whether problem solving ability belongs to the cognitive domain (see Thornton et al. 2014). Problem solving can represent spontaneous innovation or, if trial and error is involved, it may be viewed as a form of learning, in which latency to complete the task is indicative of the ability to learn (Dukas 1998; Roth et al. 2010). Even if problem solving is considered innovation, innovation could still be defined as a behavioral trait that yields changes in learned behaviors (e.g. Biondi et al. 2010) and as such likely represents some aspect of cognition.

Innovation (in the form of problem solving) has been implicated in successful colonization of novel environments (e.g. Sol et al. 2005, 2008; Wright et al. 2010) and in increased overall fitness (e.g. Cauchard et al. 2013). Problem solving ability and response to novelty are frequently the focus of studies investigating the relationship between traits that differ among individuals and may form behavioral syndromes (e.g. Webster and Lefebvre 2001; Boogert et al. 2006; Biondi et al. 2010; Cole et al. 2011, 2012).

Response to novelty, which measures an animal's response to novel stimuli, is frequently viewed as part of a behavioral syndrome or emotional state (i.e. neophobia: Sih et al. 2004), however, response to novelty can also be viewed as a form of habituation learning, where an animal's exposure to the novel stimulus allows the animal to learn that the object does not represent a threat (e.g. Roth et al. 2010). Regardless of whether cognition underlies these two behaviors, they are always recognized as important for animals inhabiting challenging or novel environments (e.g. Sol et al. 2005, 2008).

Previous findings looking at population differences in problem solving ability and response to novelty in a common garden experiment using food-caching black-capped chickadees (*Poecile atricapillus*) from the climatic extremes of their range (Alaska and

Kansas), showed significant differences in both traits (Roth et al. 2010). Chickadees from the harsher Alaska climate solved a novel problem faster and were less neophobic compared to the chickadees from the less harsh Kansas climate (Roth et al. 2010). In addition, wild-caught black-capped chickadees from more harsh winter climates have been shown to have both better spatial memory involved in recovery of food caches (they made fewer errors in recovering caches and learned the criteria faster in an associative learning task) -- especially critical in harsher environments and larger hippocampi (with more neurons) (Roth and Pravosudov 2009; Roth et al. 2011).

In food-caching mountain chickadees (*Poecile gambeli*), an environmental gradient of harsh winter conditions exists over very small spatial scales along montane elevation (Freas et al. 2012). Similar to continental variation found in black-capped chickadees experiencing differences in winter conditions mountain chickadees from harsher high elevations have superior spatial memory ability (made fewer errors in recovering caches and remembered the cache locations longer) and larger hippocampi with more and larger neurons than birds from lower elevations (Freas et al. 2012). Furthermore, these hippocampus differences remain evident even in captivity (Freas et al. 2013). The question, however, remains whether the same environmental factors associated with differences in problem solving ability and response to novelty along the large-scale climate gradient in black-capped chickadees are also associated with similar differences in mountain chickadees along the small-scale elevation gradient of winter climate severity. In other words, if problem solving ability and response to novelty are favored in harsher climates, do mountain chickadees inhabiting high and low elevations differ in these traits?

In a previous study, we failed to detect differences in neophobia between high and low elevation mountain chickadees (Kozlovsky et al. 2014), however, that study's methodology differed from that of the black-capped chickadee study (Roth et al. 2010). Here, we tested for potential elevation related differences in response to novelty using a different group of mountain chickadees from the same two elevations as our previous study, but precisely replicated the methods and novel objects following Roth et al. (2010). Furthermore, we also tested for potential sex differences in problem solving and in response to novelty.

Studies examining sex differences in problem solving and innovation have generally yielded equivocal results. In guppies (*Poecilia reticulata*), it appears that females are better innovators than males (Laland and Reader 1999). The ability to solve a novel problem in great tits (*Parus major*), on the other hand, does not appear to be associated with sex (Cole et al. 2011; Morand-Ferron et al. 2011; Cauchard et al. 2013). A study on innovativeness in meerkats (*Suricata suricatta*) reported that males appeared to be better innovators (Thornton and Samson 2012), as did a large-scale interspecific comparison of primate innovation (Reader and Laland 2001).

Research on sex differences in response to novelty in avian species has also yielded equivocal results. In studies of latency to take a novel food item, female domestic chicks were faster at taking novel food than males (Jones 1986), while in robins (*Erithacus rubecula*) and blackbirds (*Turdus merula*) no significant differences in latency to take novel food were detected (Marples et al. 1998). When exposed to a novel object, house sparrow (*Passer domesticus*) sexes displayed similar degrees of neophobia (Ensminger and Westneat 2012) while in blue tits (*Cyanistes caeruleus*) males appeared generally more neophobic than females (Arnold et al. 2007).

In light of the results of Roth et al. (2010) and the plethora of mixed results on sex differences among species with different life histories and environments, here we tested whether problem solving and neophobia differ between mountain chickadees from different elevations and between sexes. We hypothesized that mountain chickadees from high and low elevations would differ in problem-solving ability and in response to a novel object, similar to black-capped chickadees from harsh and mild environments (Roth et al. 2010), with high elevation birds solving the novel problem faster and having less neophobia toward the novel object. We additionally hypothesized that there would be no sex differences in problem solving and response to novelty as there should be similar environmental pressures acting on both sexes at different elevations.

Methods

Study subjects and capture site

Twenty-four juvenile mountain chickadees were captured at Sagehen Experimental Forest in Truckee, CA using mistnets in late November 2013 using the same elevations and exact same locations as our previous studies reporting elevation related differences in spatial memory and the hippocampus (Freas et al. 2012, 2013). Juveniles were identified based on tail feather tip characteristics as in our previous work (Freas et al. 2012, 2013; Kozlovsky et al. 2014). First year, juvenile birds were used in this study as we wanted to obtain birds that had yet to experience differential winter climates. Twelve birds (6 male and 6 female) were caught at low elevation (~1800 m) and 12 birds (6 male and 6 female) were caught at high elevation (~2400 m), separated by approximately 10 km. Blood samples were collected from all birds upon capture. Birds were brought into our housing facilities at University of Nevada, Reno where males and females were kept in separate rooms and all birds were individually housed in 42 X 60 X 60 cm cages which were visually, but not acoustically isolated. Upon arrival sex was confirmed by CLB via PCR following procedures from Fox et al. (2009). Birds were fed a diet of sunflower seeds, pine seeds, crushed peanuts, meal worms and insect pate and maintained on a winter photoperiod of 10:14 light/dark cycle. Prior to testing, chickadees were maintained in the laboratory for approximately two months and were naive to all problem solving tasks and novel objects used for neophobia testing; however, these birds were being used for mate preference experiments during the same period. Before any experiment began chickadees were habituated to waxworms.

Problem solving

Problem solving testing began in January, 2014. Problem solving ability was tested in each chickadee home cage by adapting a method developed by Z. Nemeth et al. (personal comm.). An upside-down clear test tube was baited with a waxworm and the opening was plugged with a piece of cotton (see Fig. 1). The test tube was then secured to the front of the cage with a binder clip. This set up allowed chickadees to see their reward, but in order to solve the problem chickadees had to pull out the cotton stopper allowing the waxworm to fall to the floor of the cage. Waxworms are a highly preferred food and before problem solving testing began all chickadees were eating waxworms in under a minute (in most cases it was under 30 seconds) when placed on the bottom of the cage.

Chickadees were not food deprived prior to testing and the normal diet remained in the cage during problem solving trials as the purpose of the trials were to see differences in how chickadees from different elevations and sexes spontaneously solve a problem in the face of a high-value food item. Prior to problem solving trials chickadees were given a one hour habituation period to the test tube and clip without a cotton stopper and wax worm. Problem solving trials lasted for 1 h and no more than two trials (1 in the morning and 1 in the afternoon) were conducted per day with at least 4 hours separating the two trials. Once a chickadee solved the problem it was considered successful on that trial. However, to confirm that this was not a chance solution and to look at potential learning of the problem the chickadees were given additional trials until each bird solved the problem a second time (which occurred in the following trial in all but a single low elevation bird) or until the bird reached the ceiling (occurred in a single low elevation bird). A maximum of 25 trials were used to assess problem solving abilities in each individual.

Novelty test

The novelty test began immediately following problem solving testing and was conducted in the home cage, in a feeding context following Roth et al. (2010) precisely. Both control trials and the neophobia trial were always conducted 1 trial per day on 3 sequential days at approximately the same time of day for each trial. Trials were conducted over 3 consecutive days for several reasons. Most importantly, once a bird has eaten a waxworm it may be satiated and no longer motivated to return to the feeder in subsequent trials. Second, all trials were run early in the morning (half hour after lights on) when chickadees would be the most motivated to satiate their hunger. Birds were given a pre-control trial of 300 s to assess latency to touch a familiar regular feeder on the floor, land on that feeder and take a waxworm from the feeder. The following day a novelty trial commenced with the regular feeder being replaced with a waxworm-baited, colored and spoked feeder placed in the same location as the regular feeder (exact same feeders used in Roth et al. 2010; see Fig. 2); and latency to touch the feeder, to land on the feeder, and to take the waxworm was recorded for 1800s. The spoked feeders came in 4 colors: orange, lime green, pale pink, and dark pink and feeders were assigned to individuals randomly using a random number generator. On the last of the 3 days, a post-control trial was conducted in identical fashion to the pre-control trial, to test for any carry over effects (such as increased latency to go to a familiar feeder after being exposed to a novel feeder) of the novelty trial. In all trials birds were deprived of food 30 min prior to lights off the previous day as in Roth et al. (2010).

Statistical analysis

All data were analyzed using STATISTICA computational software. Problem solving was analyzed using a General Linear Model (GLM) with sex and elevation as the between-subject variables and number of trials to solve the problem as the response variable. An interaction of elevation by sex was also included in the model. Additionally, a repeated measures GLM was used to analyze how many trials were required to solve the problem a second time once the problem was solved initially. Response to novelty was analyzed using a repeated measure GLM with novelty treatment and two controls (pre and post novelty treatment) as repeated measures, and sex and elevation as the between-subject variables. Latency to touch the feeder, land on the feeder and take a waxworm was measured for each of the three parts of the experiment (pre and post controls and well as the novelty test). This model included all possible interactions. In all cases the effect of sex was insignificant and so sex was removed from all further analyses. Lastly, we used a Spearman Rank Correlation to analyze whether the number of trials to solve the problem was correlated with degree of neophobia across both elevations, measured as the latency to take the waxworm from the novel feeder.

Results

Problem solving

When problem solving was analyzed with sex and elevation, the effect of sex was not significant ($F_{1,20} = 0.017$, $p = 0.89$) while the effect of elevation was approaching significance ($F_{1,20} = 3.95$, $p = 0.06$) and the interaction between elevation and sex was not significant ($F_{1,20} = 0.063$; $p = 0.80$). When sex was removed from analysis, the effect of elevation was significant with high elevation birds taking fewer trials to solve a novel problem ($F_{1,22} = 4.33$, $p = 0.049$; Fig. 3). The number of trials to solve the same problem the second time was significantly smaller than the number of trials to solve it for the first time (First trial – high elevation: 3.67 ± 2.01 SE; low elevation: 9.58 ± 2.01 SE; Second trial – high elevation: 1.0 ± 1.41 SE; low elevation: 3.0 ± 1.41 SE; $F_{1,22} = 13.20$, $p = 0.0015$), while the interaction between elevation and the difference in the number of trials used to solve the problem for the first and the second time was not significant ($F_{1,22} = 2.37$, $p = 0.14$).

Novelty test

When response to novelty was analyzed including sex none of the between-subject variables were significant (elevation: $F_{1,20} = 0.13$, $p = 0.72$; sex: $F_{1,20} = 1.27$, $p = 0.27$) but all birds showed significant neophobic response to novel feeders ($F_{8,160} = 14.04$; $p < 0.001$) and no interactions were significant ($p > 0.23$). When sex was dropped from analysis, the results were similar with the only significant variable being novelty treatment versus control ($F_{8,176} = 13.91$, $p < 0.0001$; Fig. 4). Planned comparisons showed that birds from both elevations did not differ significantly among the three components of each phase (touch, land, take worm) and between pre- and post-controls (Pre-control: $p > 0.98$ for all comparisons; Post-control: $p = 0.99$ for all comparisons; Pre- vs. Post-control: $p > 0.97$ for all pairwise comparisons), but differed significantly between novel object phase (neophobia) and both pre- and post-controls (touch – $p < 0.001$; land – $p < 0.001$ and take worm – $p < 0.001$ for all pairwise comparisons between all three stages of neophobia trial and both pre- and post-controls). Across birds from both elevations, there was a significant negative correlation between the number of trials it took to solve the novel problem and latency to take a worm from a novel feeder ($r_s = -0.44$, $p < 0.05$; Fig. 5); birds that solved the novel problem faster had a stronger neophobic response to the novel object.

Discussion

Mountain chickadees from high elevation solved the novel problem faster than birds from low elevation; however, chickadees from the two elevations did not differ significantly in their response to novelty. All birds reacted neophobically to the novel spoked feeder, but there were no differences in the latency to touch the novel feeder, land on the feeder or take the waxworm from the feeder between birds from the two elevations. Male and female chickadees responded similarly on both tasks, suggesting no sex related differences in problem solving ability or neophobia.

While we propose that elevation related differences in problem solving are a result of differences in innovativeness it is important to consider alternative explanations such as motivation, neophobia, motor dexterity and general activity level. Chickadees from both elevations appeared to be highly and comparably motivated to solve the novel problem. Firstly, all birds rapidly consumed the waxworm during the waxworm habituation phase. Second, when first introduced to the novel problem all birds interacted with the test tube within the first trial and in most cases within the first few minutes of that trial. Additionally, the waxworm was highly visible through the transparent test tube. Third, as birds were not deprived for the problem solving task all birds had access to their usual food *ad libitum* and as such satiety motivation should have been comparable. Forth and perhaps most importantly, there were no differences in solving the task a second time. If chickadees were differentially motivated to solve the task then this would have been the case during first and second trial in which a chickadee solved the task and not just in the initial trial. Differences in response to novelty between elevations were not found for the novelty test and, furthermore, latency to take a waxworm from the novel feeder was negatively correlated with the number of trials to solve the problem regardless of elevation. Such results suggest that the chickadees with the stronger neophobic response to novel feeder solved the problem faster than the chickadees with a lesser neophobic response. Motor dexterity has been implicated as an important component of problem solving (Griffin and Guez 2014), however, it is unlikely that this task challenged the motor dexterity of any of the birds from either location and the birds should be comparable in motor dexterity as there are no differences in head or beak morphology (DYK, CLB et al. unpublished data). Lastly, activity rate is also unlikely to have played a role in the problem solving task. Higher activity rates might yield more chance solutions to the problem than lower activity rates, however, based on our previous findings from the same populations, high elevation mountain chickadees actually have slower activity rates than low elevation chickadees (Kozlovsky et al. 2014). Overall, elevation related differences in problem solving do not appear to be explained by differences in motivation, neophobia, motor dexterity and activity rates.

The analysis comparing the first and second successful problem solving trials, suggests that learning might be involved in the problem solving process. Chickadees were faster to solve the problem the second time compared to the first time. In fact all birds, except one low elevation bird, solved the problem the second time in one trial immediately following the first successful trial. Furthermore, these two successful solutions are unlikely to be chance solutions as our observations indicate that the birds were interacting with the test tube in such a way that trial and error learning was likely occurring.

The results of the current study can be compared to a previous study of problem solving and response to novelty in hand-raised black-capped chickadees from the environmental extremes of their range (Roth et al. 2010). The problem solving results from our current study are in agreement with the problem solving differences observed in Roth et al. (2010) between Alaska and Kansas black-capped chickadees. Birds from the climatically harsher Alaska population were faster to solve a novel problem than birds from the milder climate Kansas population (Roth et al. 2010) and similarly, mountain chickadees inhabiting the harsher high elevation were faster at solving a novel problem compared to their low elevation counterparts inhabiting a milder climate.

However, unlike Roth et al. (2010), which reported that hand-reared black-capped chickadees from harsher environments were less neophobic, we detected no significant differences in response to novelty among mountain chickadees from different elevations. One possible explanation for the differences between the current study and Roth et al. (2010) is that the black-capped chickadees from Roth et al. (2010) were hand-raised in relatively restricted environments from a very young age, while the mountain chickadees from the current study were wild-caught birds, only in captivity for two months before being tested. It is reasonable to assume that birds which are hand-reared without enrichment to a wide variety of objects might be generally more neophobic than wild birds which are exposed to a wide range of objects, however, the only study to our knowledge addressing this failed to find any differences between hand-raised and wild starlings (Feenders et al. 2011). Alternatively, it is possible that mountain chickadees from different elevations, regardless of developmental environment, simply do not differ in neophobia. These results reinforce our previous conclusions based on data showing no elevation related differences in neophobia in mountain chickadees using different methods (Kozlovsky et al. 2014).

Another interesting difference between the current study and Roth et al. (2010) pertains to the relationship between problem solving ability and brain size. Many interspecific comparisons have suggested a direct relationship between general cognitive ability (e.g. a suite of correlated cognitive traits, in primates referred to as Spearman's *g*; Reader et al. 2011) and brain size (e.g. Gibson et al. 2001; Reader and Laland 2002; Sol et al. 2005, 2008; Deaner et al. 2007). Within species, black-capped chickadees from harsher environments have also been shown to have larger absolute brain size (Kozlovsky et al. 2014) in addition to better problem-solving abilities (Roth et al. 2010). In the current study, mountain chickadees from the harsher high elevation were better problem solvers than their low elevation counterparts, however, previous studies of chickadees from exactly the same elevations/populations detected no significant differences in telencephalon volume (Freas et al. 2012), which is known to be highly correlated with brain size (e.g. Krebs et al. 1989; Kozlovsky et al. 2014). Combined, these data on the same populations indirectly suggest that better problem solving ability in high elevation chickadees is not associated with differences in overall brain size. Hence, the relationship between (what we term) general cognition and brain size, at least as it pertains to problem solving, might not be as clear as suggested by some broad-scale comparisons (e.g. Healy and Rowe 2007; Chittka and Niven 2009; Pravosudov and Roth 2013). Future studies should attempt to address this relationship more directly.

Sex differences in problem solving ability have been shown for a few taxa (e.g. guppies: Laland and Reader 1999; primate: Reader and Laland 2001; meerkats: Thornton and Samson 2012), but the direction of such differences are not uniform. In avian taxa, however, available studies have failed to find sex differences in problem solving (e.g. Cole et al. 2011; Morand-Ferron et al. 2011; Cauchard et al. 2013), except with regards to social learning (Aplin et al. 2013).

A third interesting finding pertains to the correlation between problem solving and response to novelty. Based on previous research (Roth et al. 2010; Griffin and Guez 2014), we predicted that across all chickadees problem solving would be positively correlated with response to novelty. However, the results were counter to this prediction, showing a negative correlation between problem solving and response to novelty. These data are hard to interpret given the plethora of research showing a positive relationship between these two traits. Firstly, the test tube was not truly novel in that the birds were habituated to it prior to testing. It is also possible that the problem solving task and the response to novelty task differ in the level of neophobia generated. It is possible that the brightly colored spoked feeders were considered a much more threatening novel stimulus than the transparent test tubes with silver wire wrapped around it. Alternatively, chickadees are hesitant about going to the ground in the wild and the floor of their cages, as this represents a potential predation risk and so the fact that the novel feeder was on the ground while the test tube was clipped to the cage near one of their perches could potentially explain the correlation observed. It would then be expected that chickadees would take longer to take the waxworm from the ground in the absence of the novel feeder. It is also possible that neophobilia is responsible for approaching the novel problem while neophobia is responsible for reduced latency to feed from the novel feeder, as it has been suggested that these two traits can vary independently (Griffin and Guez 2014). Whether one of the aforementioned factors or some unknown factor is driving the relationship observed here remains a topic for future investigation.

Studies on sex differences in response to novelty, even within Aves, have also yielded equivocal results (e.g. domestic chicks: Jones 1986; blackbirds and robins: Marples et al.

1998; house sparrow: Ensminger and Westneat, 2012; blue tits: Arnold et al. 2007). Here, we failed to detect significant sex differences in problem-solving ability (a measure of innovation) or response to novelty (a measure of habituation) in mountain chickadees from different elevations. Sex differences are suggested to evolve over a long evolutionary time scale and require very strong differential selection pressures to evolve (Sherry et al. 1992), but male and female mountain chickadees at different elevations appear to experience similar selection pressures. However, if these traits change as a result of phenotypic plasticity, these results do not support the “necessity hypothesis” which would predict that female mountain chickadees would be better innovators than their male counterparts since they are less competitive at obtaining food. These results agree with the findings that sex was not associated with differences in innovativeness of great tits (e.g. Cole et al. 2011; Morand-Ferron et al. 2011). While the “necessity hypothesis” does not directly suggest differences in response to novelty, based on the logic of the hypothesis, one might expect female chickadees to be less neophobic as well, which was also not supported here. Even though the effect of sex on both problem solving and neophobia were highly non-significant, it is important to add that our sample size was fairly small and so it remains possible that our study lacks sufficient statistical power to detect significant differences.

While the impetus for this study was Roth et al (2010) which used hand-raised black-capped chickadees taken from the nest around 10 days of age, the role of plasticity in shaping the differences in problem solving cannot be ruled out in the current study. With regards to spatial memory and the hippocampus, all indirect evidence points towards selection driving the observed differences (differences persisting in a common garden experiment (Freas et al. 2013), no significant differences in developmental trajectories (Kozlovsky et al. 2015, Ch. 2); elevation specific female mate preference (Branch et al. 2015). For problem solving, however, it is impossible to rule out any potential differences with prior experiences in mountain chickadees, even though in black-capped chickadees such differences existed in birds hand reared and maintained in the same lab environment from 10 days of age (Roth et al. 2010). In this study, juvenile birds were used prior to experiencing winter climate differences, which is the main selection event for juvenile chickadees (Freas et al. 2012). Additionally, birds were housed in identical conditions in captivity for two months prior to any testing and as such, immediate environmental effects are unlikely to produce the resulting differences in problem solving. During the breeding season, climate and food availability between the two elevations appear to be similar although data on are lacking. Potential differences in food availability or foraging locations, however, could potentially lead to selection or learned differences, which may translate into differences in problem solving ability between chickadees from different elevations.

In conclusion, mountain chickadees, like black-capped chickadees, show environment related differences in in problem-solving ability, with better problem-solving ability associated with harsher environments. Neophobia in mountain chickadees, however, did not differ between the two elevations, which suggests that population variation in problem solving ability might exist without corresponding variation in neophobia, even though in our case the two traits appear to correlate in a negative direction across all individuals tested (with a longer latency to feed from the novel object being associated with fewer trials to solve the problem). Such findings are counter to the idea that neophobia and problem solving are fundamentally linked in a positive direction (e.g. Greenberg 2003). In addition, no significant sex differences existed among mountain chickadees in either problem solving or neophobia. Overall, our results are consistent with the idea that living in harsher environments might select for increased problem solving ability in chickadees, whether over a large or small-scale, yet decreased neophobia does not seem to be specifically associated with harsher environments or with variation in problem solving ability.

Acknowledgments

We would like to acknowledge Elena Pravosudova for help capturing birds and Shelby Brown, Frank Gonzalez, Kallie Kappes, and Emily Weissgerber for help with bird care and maintenance and University of Nevada, Reno's EECB doctoral program for research funds. All authors were supported by NSF (IOS-1351295) to VVP.

Ethical standards

All experiments reported here comply with the current laws of the United States of America. Chickadees were collected under Federal (MB022532) and California (5210) scientific collecting permits. All animal procedures were in accordance with the University of Nevada Reno Animal Care and Use Protocol (00576)

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Figure Legends

Fig. 1

Photograph of one of the problem solving apparatuses. Note that there is a mealworm inside the apparatus for display rather than the waxworms that were used during the actual experiment

Fig. 2

Photograph of the four novel feeders used for the response to novelty trial

Fig. 3

Number of trials to solve a novel problem in chickadees from high and low elevations. Sexes are pooled and bars represent SE

Fig. 4

Latency to touch a feeder, land on a feeder and take a waxworm from a feeder in pre-control (pre), neophobia (neo), and post-control (post) trials in chickadees from high (closed circles) and low elevations (open circles). Sexes are pooled and bars represent SE

Fig. 5

The relationship between the number of trials to solve a novel problem and the latency to take a worm from a novel feeder

Fig. 1

Fig. 2

Fig. 3

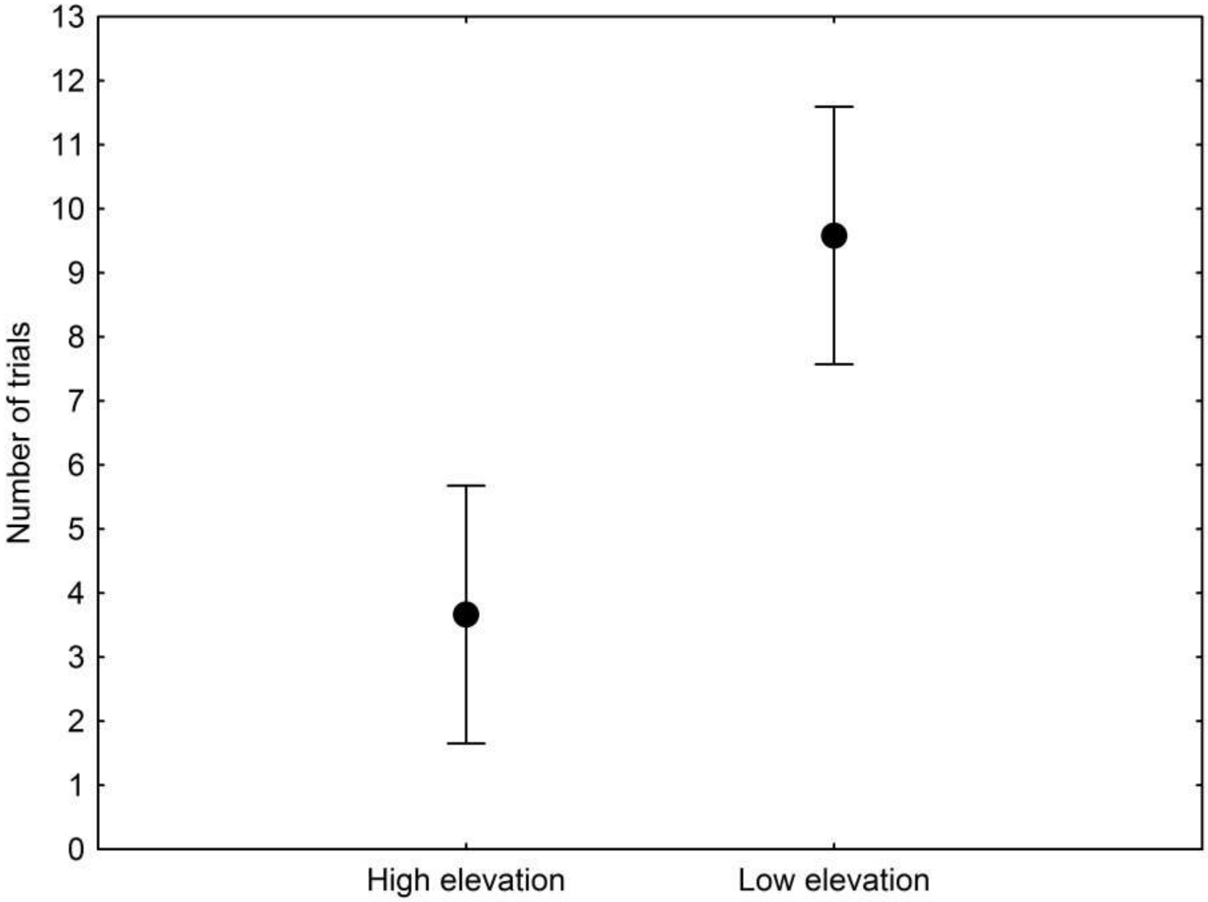

Number of trials
0
1
2
3
4
5
6
7
8
9
10
11
12
13
High elevation
Low elevation

Fig. 4

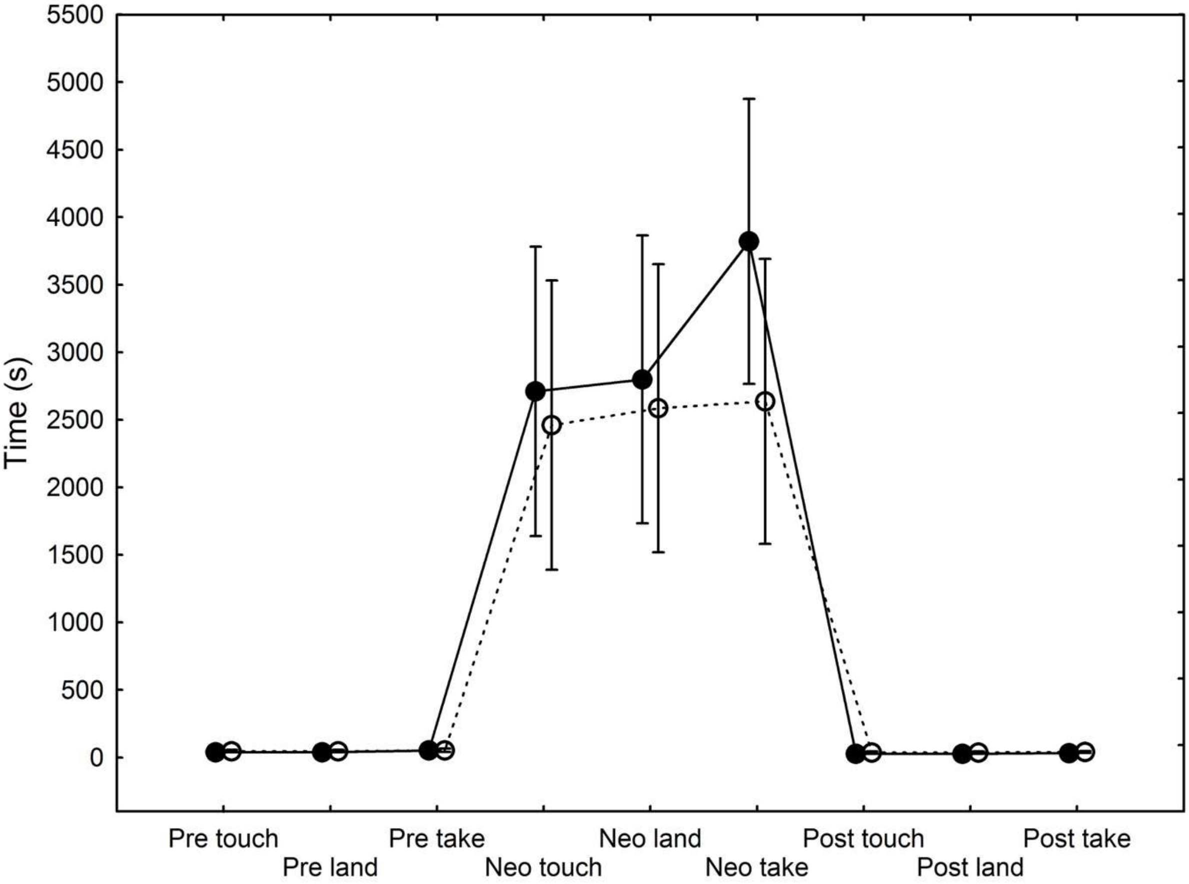
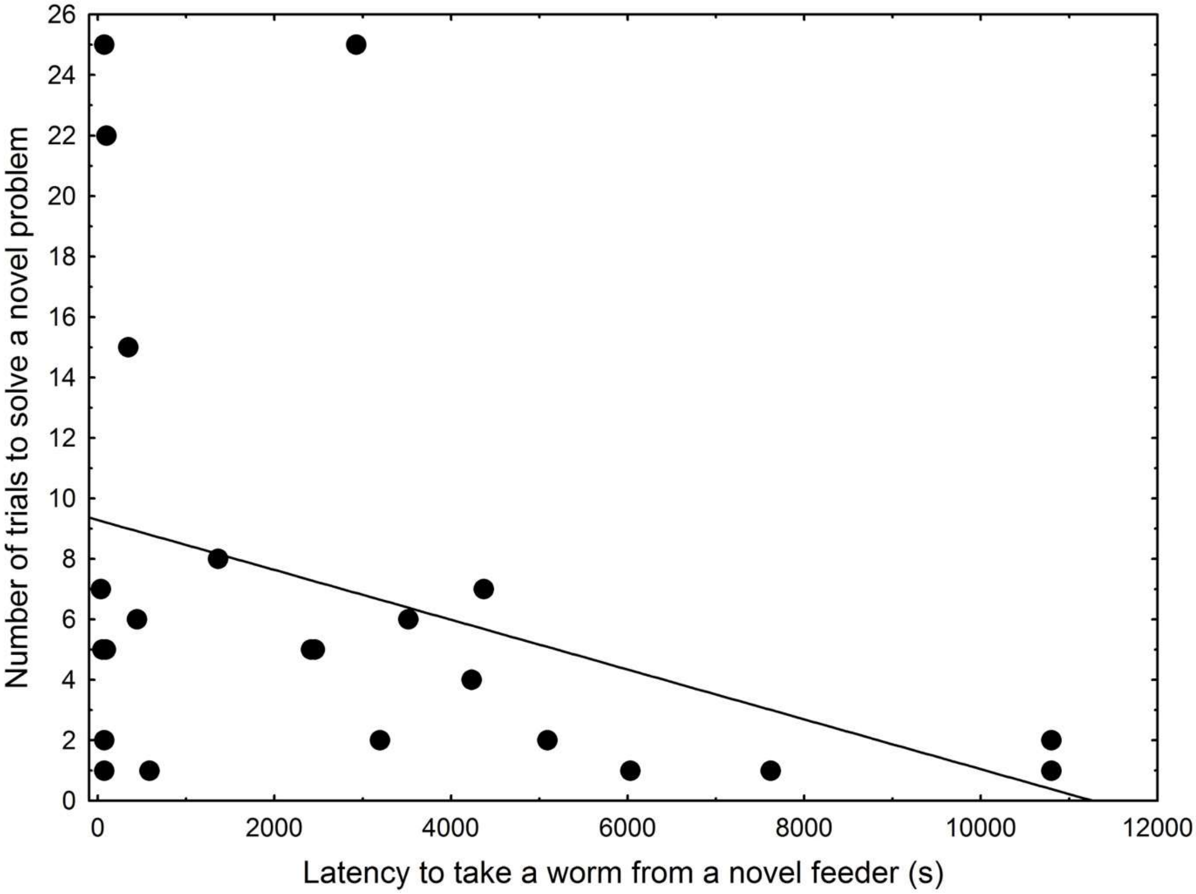

5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Time (s)
Pre touch
Pre land
Pre take
Neo touch
Neo land
Neo take
Post touch
Post land
Post take

Fig. 5


Number of trials to solve a novel problem
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Latency to take a worm from a novel feeder (s)
0
2000
4000
6000
8000
10000
12000

Ch. 2: Elevation related differences in parental risk taking behavior are associated with cognitive variation in mountain chickadees

Dovid Y. Kozlovsky, Carrie L. Branch & Vladimir V. Pravosudov

Abstract

Breeding animals must balance their current reproductive effort with potential costs to their own survival and consequently to future reproduction. Life-history theory predicts that variation in reproductive investment should be based on fecundity and life expectancy with longer lived species favoring their own survival over parental investment. Recently, variation in parental risk taking was also linked with differences in cognition suggesting a trade-off between cognitive ability and risk taking. Here, we tested whether mountain chickadees from two different elevations with known differences in cognitive ability differ in their parental risk taking by comparing the responses of nesting birds to a potential predator. Higher elevations are associated with shorter breeding season limiting re-nesting opportunities, but chickadees at high elevations also have better cognitive abilities, which might be potentially associated with better survival. Compared to lower elevations, high elevation chickadees laid larger clutches, showed longer latencies to return to the nest in the presence of a hawk, had lower fledging success and exhibited higher rate of complete nest failures. Nestling development among successful nests, however, was similar between elevations. These data are not consistent

with life history hypothesis because birds at high elevation invest more in clutch, while at the same time take less risk when facing potential danger to themselves, which could jeopardize their current reproductive success. These data, however, are consistent with the hypothesis that better cognitive abilities might be associated with less parental risk taking.

Introduction

Breeding animals must partition their resources between investing in current reproductive effort or in their own survival, especially when facing increased risk of mortality (Williams 1966; Trivers 1972; Ghalambor & Martin 2000). According to life history theory, differences in parental effort should be reflected by parental risk taking when a parent could either provide parental care at a potential risk to its own survival or reduce its parental effort to increase its own probability of survival (Ghalambor & Martin 2000, 2001; Ghalambor et al. 2013). In line with this theory, low fecundity and longer life span should be associated with parents favoring their own survival over parental investment in their current offspring (Curio 1988; Ghalambor & Martin 2000, 2001). For example, longer-lived less fecund species have been hypothesized to invest less in current offspring in favor of self-survival and future reproduction compared to shorter-lived, more fecund species, which are expected to do the opposite (Trivers 1972; Linden & Moller 1989; Mauck & Grubb 1995; Ghalambor & Martin 2000, 2001). Similarly, the potential to successfully renest during the same season, which could be hypothetically expected in populations with a longer breeding season (e.g. Bears et al. 2009), might also be predicted to be associated with a reduction in parental effort, especially in short-lived species. When there is only a single chance of reproduction within a season, individuals could be expected to favor investment in their current reproduction over future reproduction (e.g. Badyaev & Ghalambor 2001), especially when the probability of survival until next season is relatively low. When breeding season is sufficiently long to accommodate multiple reproductive events, individuals might be expected to favor their own survival, especially during the first reproduction of a given breeding season. For example, northern birds limited to a single reproductive event per year have been reported to suppress physiological stress response in the face of inclement weather that might ultimately result in their death while their more southern conspecifics do show such response and abandon reproduction in favor for later re-nesting (Wingfield et al. 1995; Silverin et al. 1997; Silverin & Wingfield 1998).

Recently, it has also been suggested that variation in at least some components of parental effort might be associated with the evolution of cognition and that better cognitive ability might be linked with reduced risk taking in parents, which might have a negative effect on reproductive success. Cole et al. (2012) recently reported that individual great tits (*Parus major*) with better problem solving ability laid larger clutches, but also deserted nests more frequently than their counterparts who were unable to solve the presented problem. Nest abandonment resulted in no overall fitness advantage for problem solvers. It appears that better cognitive ability of problem solvers allowed them to produce more offspring, but reduced parental effort may have negated such an effect via higher nest desertion rates. Cole et al. (2012) suggests that birds with enhanced cognitive ability deserted their nests, as a result of human disturbance when handling adults that had young chicks that were not entirely viable. This result potentially points to a parental risk taking strategy resulting in nest abandonments.

The trade-offs between cognitive ability and reproductive effort might potentially be based on two different processes. First, it has been suggested that enhanced cognitive ability in animals is associated with a longer lifespan (e.g. Sol et al. 2007) by increasing the probability of survival via increased foraging efficiency in unpredictable environments (e.g. Roth et al. 2010). The life history trade-offs hypothesis then might predict that animals with superior cognitive ability, which live longer, might invest less in current offspring in favor of future reproduction relative to less intelligent, shorter-lived animals. Alternatively, enhanced cognitive ability may reflect a direct trade-off with other life history traits related to fitness if enhanced cognition can only be achieved by compromising some other traits (Cole et al. 2012; Healy 2012; Kotrschal et al. 2013). In other words, enhanced cognitive ability may be associated with reduced parental risk taking as part of a behavioral syndrome (e.g. van Oers et al. 2004; Carere & Locurto 2011) in which case parental investment strategies might differ from those predicted by life history based on longevity and fecundity.

Here we compared responses of breeding mountain chickadees (*Poecile gambeli*) from high and low elevations to a potential predator for adults to assess parental risk taking behavior and hence at least one component of parental effort. We used two elevations which have been under intense investigation, allowing a comparison of potential differences in risk taking with previously established differences in spatial memory (Freas et al. 2012, 2013) and problem solving (Kozlovsy et al. 2015, Ch. 1).

Returning to the nest in the presence of a predator is likely associated with increased risk for the parents (Ghalambor & Martin 2000, 2001), but delayed nest attendance could have negative effects on reproductive success via its effect on incubation patterns or offspring feeding. Shorter latencies to enter the nest with increased risk for the parents would indicate higher parental effort associated with more risk taking at a potential cost to parents own survival (Ghalambor & Martin 2000, 2001). To control for potential differences in immediate environmental conditions, we also used a control – a dove model, which represents a non-threatening bird stimulus.

Mountain chickadees at different elevations provide a good case to test which of the three previously described evolutionary processes might generate potential differences in parental risk taking behavior. First, at our study area, chickadees at higher elevations experience longer periods of snow cover in the spring and receive snow fall sooner in the autumn, resulting in a significantly shorter breeding season with birds from high elevations less likely to succeed at re-nesting. Such differences in re-nesting opportunities might be associated with chickadees at higher elevations investing more in their current reproductive effort at an increasing risk to their own survival. Second, we have previously demonstrated that chickadees at high elevations cache more food, have significantly better spatial learning ability associated with successful recovery of food caches, have significantly different morphology of the hippocampus and have better problem solving ability (Freas et al. 2012; Freas et al. 2013a, 2013b; Kozlovsky et al. 2015, Ch. 1), traits that might potentially result in higher survival probability at high elevations. If so, it might be expected that chickadees at high elevations should invest less in their current reproduction and exhibit less risk taking behavior. Finally, better cognitive abilities in high elevation chickadees might be associated with reduced risk taking behavior as a trade-off resulting from selection on cognition associated with harsher winter climate at higher elevations demanding heavier reliance on food caches for survival (Freas et al. 2012). If that were the case, only parental risk-taking behavior might be affected, while other components of parental effort might reflect other life-history processes, and significant and persistent differences in cognition associated with elevation allow investigation of potential trade-offs without testing cognitive abilities in individuals.

We also compared nesting success and fledgling development (e.g. clutch size, brood size, fledgling mass, feather length, and within nest variation in development) to assess potential differences that might be associated with expected differences in parental risk taking and parental investment. If parental strategy in high elevation chickadees is associated with reduced breeding season, chickadees at high elevations should lay larger clutches and take more risks to insure successful reproduction. If parental strategy at high elevation is associated with increased survival, chickadees at high elevations should lay smaller clutches and take fewer risks, which would make future reproduction more likely. Finally, if selection on better spatial learning and problem-solving abilities at high elevations is associated with personality trade-off (e.g. better learning is linked with less risk taking), chickadees at high elevations should lay larger clutches (because of their superior ability to cache food and retrieve caches), but exhibit less parental risk-taking behavior, which might result in reduced overall breeding success

Methods

Data were collected at Sagehen Experimental Forest in Tahoe National Forest just north of Truckee, California at two elevations (ca. 1800 m – low elevation and ca. 2400 – high elevation) used in our previous work showing significant elevation related differences in spatial memory and hippocampus morphology (Freas et al. 2012; Freas et al. 2013a, 2013b). We specifically used these established elevations because of the previous work establishing differences in memory and brain morphology even though comparison of just two elevations unfortunately limits the scope of the study. Variation in elevation within the two established main elevations, however, was extremely small. Established nestboxes were monitored daily starting on May 2, 2013 to determine the start date of egg laying, the beginning of incubation, clutch size and brood size at day 16.

Mountain chickadees are secondary cavity nesters and do not excavate their own nests (Mccallum et al. 1999). We only used nestboxes in our study and so had no information about any nests in natural cavities; thus we could not estimate true nesting density at our study site. We tested chickadee responses during two nesting stages – during incubation (only female incubates) and during nestling provisioning by both males and females (Mccallum et al. 1999). As responses to the predator might vary depending on nesting stage, we tested all birds at the same time during their nesting and carefully monitored all critical nesting dates and events.

When the first egg was found the day was recorded as egg day 1 for that nest. When incubation had started clutch size and egg mass were recorded for each egg and that day was considered incubation day 1. The day a nest had any eggs hatched was recorded as nestling day 1. On nestling day 16 all surviving nestlings were counted as the final brood size and nestling mass, length of left and right outermost primaries and outermost rectrices (to estimate fluctuating asymmetry) were measured. Following nestling measurements nests were monitored every other day for fledging day, in order to determine the length of the nestling stage. Finally, we analyzed within-nest coefficient of variation (CV) for all of the developmental parameters, as higher within-nest CV would indicate less favorable developmental conditions with large variation in nestling developmental condition.

All model presentation experiments during incubation (n = 16 nests at low elevation and n = 11 nests at high elevation) started between days 5 and 8 of incubation. We used a mounted sharp-shinned hawk (*Accipiter striatus*) specimen (sharp-shinned hawks are common in low densities at both elevations and appear to be primary avian predators for mountain chickadees; Mccallum et al. 1999) as a predator for adults and a dove model as a generic, non-threatening bird-control of a similar size. Both models were attached to a branch and placed on a tripod set at the height of nestboxes. All birds exhibited strong mobbing behavior directed at the hawk, but not the dove model, which suggests that chickadees perceived the hawk model as a predator. Pilot data showed that mountain chickadees did not react adversely to the tripod by itself. Order of presentation (e.g. hawk or dove) was determined randomly by coin-flip and only one model was presented to each nest on a given day at the same time of day. In all but four nests, models were presented on two consecutive days. Due to rain, one day elapsed between presentations in the other four nests.

Model presentations for the incubation period began when a female left the nestbox and the tree with the nestbox after a bout of incubation. Upon the female leaving the nest, the tripod was immediately placed 5 m from the nestbox with the hawk or the dove model in front of the nest and facing the nestbox entrance. At both elevations models were placed in clear view of the nest in a similar fashion so that birds returning to the nest would have a similar view of the models. Bird behavior upon returning to the nest was observed through binoculars by DYK from 35 to 50 m away from the nestbox. Observer location was chosen so that the observer was as inconspicuous as possible, while maintaining a view of the nestbox and surrounding area. Chickadees did not appear to react to the observer being at the aforementioned distance and location. Once an observer location was chosen it was used for both hawk and dove presentations during both incubation and nestling stages (see below). Presentations ended and models were removed when the female went into the nestbox for a minimum of 5 min, indicating the start of a new bout of incubation or after 50 min if females did not enter the nest (which happened at one nest at low elevation and 4 nests at high elevation). Latency to enter the nestbox was recorded for all nests. Male chickadees do not incubate and never attempted to enter the nest during the experiment.

Model presentations during the nestling feeding stage (n = 12 nests at low elevation and n = 12 nests at high elevation) started between nestling day 5 and 8. Due to different logistical reasons, we tested different number of nests during nestling feeding stage and during incubation. Presentations during the nestling stage were conducted in the same fashion as during incubation with one exception. Models were presented when a parent (either male or female) had just left the nest after feeding the chicks and the second parent was greater than 20 m from the tree with the nestbox and actively foraging. When the second parent was within 20 m of the nestbox tree, model presentation did not begin until both parents had fed the chicks and left the tree with the nestbox. In most cases, both parents came to the nestbox simultaneously and so presentations occurred after both parents had fed the chicks and left. Latency to enter the nestbox by any parent (which ever was the first) was again recorded for all nests. Birds were not individually marked and so we could not determine whether male or a female entered first, but we assume that any delay to enter the nest, regardless of potential gender differences, represent parental risk taking behavior. Presentations ended and models were removed when either of the parents went into the nestbox or after 30 min if either of the parents did not enter the nest (which happened at two nests at low elevation and 5 nests at high elevation).

Statistical analyses were conducted using STATISTICA software. We tested for specific effects associated with elevation and with model type and so all analyses used mixed General Linear Models (GLM) with model or control as the repeated measure and elevation as the between-subject variable. In all cases the response variable was latency to enter the nestbox and the GLM tested whether such latency was significantly associated with elevation and model type. As we had specific expectations that there should be significant differences between elevations specifically during the hawk, but not during the control (dove) presentations, we used planned comparisons to test for specific elevation effects whether there was a significant elevation by treatment interaction. To test for potential elevation related differences in all nesting parameters (e.g. clutch size, number of fledglings, fledgling mass) we used general linear models with elevation as the predictor variable and a nesting parameter (using nest mean as a sampling unit) as the response variable for each analysis. For means, but not for CVs, clutch or brood size was used as a covariate to control for any potential variation in clutch or brood size. The sample size is different for different parameters due to some nest failing at different nesting stages. Variables that did not meet normality assumptions (e.g. hatching and fledging success) were log-transformed.

Results

First, we analyzed only the nests that were used during both nesting stages in the same model (Mixed General Linear Model with nest and nesting stage as repeated measure variables and elevation and model type as independent variables). In this analysis, elevation ($F_{1,21} = 4.32$, $p = 0.05$), nesting stage (incubation vs nestlings; $F_{1,21} = 11.05$, $p = 0.003$), and model type (hawk vs control; $F_{1,21} = 75.21$, $p < 0.0001$) were significant predictors for the latency to enter the nest (Figures 1, 2). There also was a significant interaction between elevation and model type ($F_{1,21} = 8.67$, $p = 0.008$), but not between elevation and nesting stage ($F_{1,21} = 0.01$, $p = 0.92$). Planned comparisons showed that there were no significant elevation differences in response to the dove model (p's > 0.5), but responses to the hawk model differed significantly (p's < 0.05) with chickadees at high elevations taking significantly longer to enter the nest in the presence of the hawk during both incubation and nesting periods (Figures 1, 2).

Combining all data resulted in dropping several tested nests (4 from low elevation and 1 from high elevation) from the analyses as not all nests were used during both incubation and nestling stage due to various logistical reasons. When nests were analyzed separately for incubation and nestling stages to take advantage of larger sample sizes, the results were essentially the same. There was a significant difference in latency to enter the nestbox between the hawk and the dove model presentation during the incubation stage with birds from both elevations taking more time during the hawk presentation

($F_{1,25} = 35.80$, $p < 0.001$, Figure 1). There was a significant elevation by model type interaction ($F_{1,25} = 6.74$, $p = 0.02$) even though elevation was not a significant general predictor for the latency to enter the nest during the incubation stage ($F_{1,25} = 2.24$, $p = 0.15$). Planned comparisons showed high elevation chickadees took significantly more time to enter the nest during the hawk model presentations compared to low elevation chickadees ($p = 0.006$), but there were no significant elevation related differences in the latency to enter the nest during the dove model presentation ($p = 0.5$). During the nestling feeding stage, there was a significant difference in latency to enter the nestbox between the dove and the hawk presentations for birds from both elevations ($F_{1,21} = 27.15$, $p < 0.001$, Figure 2). There was a significant effect of elevation ($F_{1,21} = 4.67$, $p = 0.04$, Figure 2) on the latency to enter the nest with higher latencies for the high elevation birds, but an interaction between elevation and model type was not statistically significant ($F_{1,21} = 2.76$, $p = 0.11$). Planned comparisons following specific a priori predictions confirmed that the general elevation effect was driven only by the response to the hawk model. Compared to low elevation chickadees, it took significantly more time for high elevation birds to enter the nest during the hawk presentation ($p = 0.01$), but the latency to enter the nest did not differ significantly between chickadees from high and low elevations during the dove presentation ($p = 0.87$).

High elevation chickadees laid significantly larger clutches than low elevation chickadees (Table 1) with date of the first egg as a significant covariate ($F_{1,51} = 12.98$, $p < 0.001$); later nests had smaller clutches. In contrast, there were no significant elevation related differences in brood size (Table 1) with date of the first egg having no significant effect as a covariate ($F_{1,38} = 0.11$, $p = 74$). Fledging success (proportion of young fledged/clutch size), on the other hand, was significantly lower at high elevation (Table 1), which potentially explains elevation differences between clutch size and brood size. The proportion of nests that completely failed prior to nestling age of 16 days was also significantly higher at high elevation (38% failed nests at high elevation based on total of 21 nests vs 14.7% failed nests at low elevation based on total of 34 nests; $x^2 = 3.93$, df=1, $p = 0.047$). In contrast, among the nests that succeeded (e.g. fledged at least 1 nestling or more), there were no significant differences in any of the measured developmental parameters between high and low elevation mountain chickadees whether using nest means or within nest coefficient of variation (Table 1). Differences remain statistically non-significant even when we did not control for clutch or brood size.

Discussion

Our results showed that chickadees from high and low elevations differed significantly in their parental risk taking behavior in response to an elevated risk to the parents near the nest. High elevation chickadees took significantly longer to enter the nestbox in the presence of the hawk model, but not the dove, during both incubation and nestling feeding stages compared to birds from low elevation. Interestingly, high elevation chickadees also laid larger clutches, but fledged a similar number of young compared to low elevation birds due to significantly lower fledging success. Most importantly, the differences between clutch size and brood size appear to be related to nest failures rather than just to brood attrition. Proportion of complete nest failures was significantly higher at high elevation, but fledgling development indices in nests that succeeded were not statistically different between elevations suggesting that parents at high and low elevations were similarly capable of rearing young. Overall, our data showed that elevation related differences in latency to enter the nest in the presence of the hawk is negatively associated with fledging success. One limitation of our study is that it focused on birds from just two elevations, but, at the same time focusing on specific elevations that have been under intense investigation for a long time (e.g. Freas et al. 2012, 2013; Kozlovsky et al. 2015, Ch. 1) allowed comparisons with multiple traits, most importantly cognition (spatial memory and problem solving).

Our results suggest that high elevation birds favor less parental risk taking and invest in their own survival over investing in their current offspring more than their conspecifics from lower elevation. When these birds faced increased risk of predation associated with attending the nest, they showed more hesitation, compared to the low elevation chickadees, which would likely increase their probability of survival at a potential cost to their current reproductive success. Low elevation birds, on the other hand, appear more willing to take risks to invest in their current reproduction at a potential cost to their own survival.

Within elevations, latency to attend the nest in the presence of the predator had no significant association with either hatching or fledging success. Considering that the largest differences were present specifically between elevations, such result was not surprising as the variance within elevations was smaller than the variance between elevations. In addition, much larger sample sizes might be required to detect such association on an individual level, as it might not be expected that a single presentation of a predator will immediately have significant consequences. At the same time, elevation related association was significant for both latency to attend the nest in the presence of the predator and fledging success – high elevations were characterized by longer latencies, lower fledging success and higher total nest failure rate.

It is important to note that chickadees expressed mobbing behavior when exposed to the hawk model and in some cases, mobbing might be considered a risky behavior. If that were the case, chickadees at high elevations could be considered engaging in more risky mobbing. We, however, do not think that in our case, mobbing should be considered more risky than entering the nest. First, sharp-shinned hawks are predominantly an ambush predator for small birds (Bildstein & Meyer 2000) that rely on surprise attacks. We could not find any published information on whether sharp-shinned hawks can be successful or even attempt to attack birds when mobbed while perched in the open. Second, when chickadees mobbed the hawk model, they always remained under cover in tree canopy where the risk of being killed by a hawk is likely very low. Finally, chickadees from both elevations exhibited the same mobbing behavior – the only difference was that it took longer to enter the nest at high elevations. In these cases, mobbing always attracts many other birds that could increase vigilance, diffuse the individual risk of predation, and help drive the predator away, yet the cost of mobbing avian predators are not well documented – except that birds engage in mobbing instead of doing something else, like attending the nest, which is likely the main cost. Flying to the nest, on the other hand, requires leaving protective cover and hence likely represent a much higher risk. Most importantly, not attending the nest in the presence of the hawk likely represents an important cost that might negatively affect reproductive success.

Our results are not fully consistent with predictions based on life-history theory associated with shorter breeding season at high elevations (see Figure 3). High elevation chickadees start breeding on average 2 weeks later than low elevation chickadees despite being in close geographic proximity (ca. 10 km) due to differences in climate (Table 1). Winter also starts earlier at higher elevation, which, combined with a later breeding start provides a shorter reproductive season at higher elevation and might reduce the probability of re-nesting if first nesting attempts fail. Lower elevation birds, on the other hand, have a longer breeding season and are more likely to succeed at the second breeding attempt if the first one failed. Given such differences between high and low elevation, it might be hypothesized that high elevation chickadees should invest more in their current reproduction then their conspecifics from lower elevation because they might not be able to re-nest. Parental investment in larger clutch size at high elevation is consistent with this hypothesis, yet our results on parental risk taking are directly opposite to the predictions – compared to low elevation, chickadees at high elevation were more cautious when in presence of the hawk, which might have negative effect on current reproduction. Supporting this idea is the fact that fledging success was indeed lower and total nest failure rate higher at high elevation.

Our data are also not fully consistent with the idea that elevation related differences in parental risk taking might be associated with potential differences in probability of overwinter survival (see Figure 3). It has been suggested that greater cognitive abilities might be associated with increased survival probabilities (Sol et al. 2007) and increased survival might be expected to be associated with a decreased investment in current offspring in favor of increased investment in future offspring. If parental investment strategy in high elevation chickadees were based on higher survival probability due to their superior cognition, it would be expected that compared to low elevation, chickadees from high elevation should lay smaller clutches in addition to taking less risks. Investing in larger clutches provides additional parental cost, which can be avoided when probability of survival is high (e.g. Trivers 1972; Linden & Moller 1989; Mauck & Grubb 1995; Ghalambor & Martin 2000, 2001). While being more cautions in the presence of the predator (less parental risk taking) is consistent with this hypothesis, significantly larger clutch sizes at high elevations are not. Unfortunately, neither Cole et al. (2012) nor our study have the data on potential differences in survival between birds with cognitive differences and future studies should attempt to address this gap. Overall, however, larger investment in clutches but lower parental risk taking in high elevation chickadees are not consistent with the life history theory based on potentially longer life span.

Our data, on the other hand, appear to be consistent with the hypothesis that enhanced cognition might be associated with cognition-parental-risk-taking trade-off suggesting that better cognitive abilities might be associated with some costs affecting reproduction (see Figure 3). Chickadees from high elevation with significantly better spatial learning ability associated with large morphological differences in the brain (Freas et al. 2012) and better problem solving ability (Kozlovsky et al. 2015, Ch. 1) laid larger clutches, possibly due to their superior cognition increasing foraging abilities, but had significantly lower fledging success associated with less parental risk taking during both incubation and rearing nestlings. Significantly lower fledging success appears to be mainly due to much higher rates of complete nest failure. Yet among the successful nests, young from high and low elevations appear to develop similarly suggesting birds at high elevation are just as capable of rearing young as birds at lower elevations, but they are less prone to risk taking resulting in higher frequency of complete nest failures. Thus, different components of parental investment show the opposite patterns: on one hand, high elevation chickadees invest more into their clutches, but on the other hand, they appear willing to jeopardize their breeding success by being more cautious in the presence of the predator. These data are therefore consistent with the trade-off hypothesis suggesting enhanced cognitive ability might be associated with a reduced parental risk taking across different contexts of perceived risk, suggesting a trade-off between cognition and parental investment strategy (e.g. Healy 2012).

It remains possible that the link between parental risk taking and elevation might be mediated by other correlated traits not associated with cognition. For example, it has been suggested that more aggressive individuals have a greater dispersal tendency (e.g. Duckworth & Badyaev 2007), but also provide less parental care (Duckworth 2006). However, mountain chickadees at our high elevation are less aggressive than the low elevation chickadees (Branch et al. 2014), which is inconsistent with this hypothesis.

All of the above-discussed hypotheses imply that differences in behavior (both cognition and parental risk taking) have been produced by natural selection and are likely based on some heritable mechanisms. Our previous data on elevation related differences in cognition and the brain all indirectly suggest that such differences have been produced by differential selection pressures associated with different environmental conditions (Freas et al. 2012). While we do not see genetic population structure based on neutral genomics (Branch et al. 2017), we do not yet have a genetic basis of elevation related differences in behavior. Chickadees in our study were tested in different environments (high and low elevations) and so it might be possible that differences in parental responses to increased predation risk might be due to some immediate environmental differences.

For example, it is potentially possible that birds at higher elevation have better foraging success either because they are better foragers or because of more abundant food, in which case they can afford to come to the nest at longer time intervals when feeding young. However, this explanation appears unlikely. First, high elevation chickadees laid larger clutches but also experienced higher rates of complete nest failures. At the same time developmental indices for young in successful nests did not differ significantly between elevations, which argues against better foraging conditions. Second, the same pattern with high elevation birds taking longer to enter the nest was present during both incubation and feeding young. Delaying entering the nest during incubation can lead to negative egg cooling effects (e.g. Olson et al. 2006), which are independent of foraging success and are not likely to be compensated for later. If a female can feed more efficiently she would actually be expected to return to the nest after a shorter rather than a longer interval (e.g. Chalfoun & Martin 2007). In addition, if chickadees from high elevation feed the young at longer intervals, it would be detectable during the dove presentation (dove was still a novel, albeit a non-dangerous object). Yet birds from different elevations did not differ in their latency to return to feed the young during the dove presentation, but exhibited significant differences specifically during the hawk presentation. Interruptions in feeding of rapidly developing nestlings are not likely to be compensated for later without any negative effects and so delaying feeding presents a risk to current reproduction (e.g. Konarzewski et al. 1996; Searcy et al. 2004).

Overall, the lack of differences found in response to the dove model generally argues against potential effect of immediate ecological conditions, otherwise birds from different elevations should differ in their response to the dove model as well. There were also no significant differences in brood size between high and low elevations, which also argues against the idea that parents at high elevation might not need to attend the nest as frequently as birds from low elevation. Differences in foraging success, on the other hand, would be expected to produce the opposite outcome to predator presentation during incubation and during young feeding. Considering that we found no significant differences in brood size due to higher young attrition at high elevation, this explanation for higher latencies to return to the nest in the presence of the hawk specifically at high elevation appears unlikely.

Our finding that chickadees lay larger clutches at high elevations is in contrast to many previous studies suggesting that birds lay smaller clutches at high elevation (e.g. Kremetz & Handfored 1984; Badyaev, 1997; Badyaev & Ghalambor 2001; Fargallo 2004; Lu et al. 2010). Yet, some previous reports showed no elevation differences in clutch size (e.g. Hamann et al. 1989; Bears et al., 2009) and still others reported that birds lay larger clutches at high elevations (e.g. Dunn et al. 2000; Camfield et al. 2010). Larger clutches produced at high elevation might suggest potentially better foraging conditions at our field site even though it is generally thought that food availability is lower at high elevation (e.g. Bears et al. 2009; Lu et al. 2010), but then it might also be expected that these birds should raise larger broods and have faster developing young, which was not the case. The fact that high elevation chickadees laid larger clutches but raised smaller broods, similar in size to those from low elevation, and had significantly higher rate of complete nest failures is more in line with the cognition-reproductive effort/parental risk taking trade-off hypothesis. The ability to lay more eggs could be related to the birds' ability to be successful foragers via better spatial learning ability rather than to greater food abundance. Overall greater food abundance would have allowed high elevation birds to successfully fledge more young, but reduced brood size and higher rate of nest failures suggests less risk taking resulting in reduced parental effort.

Our experiment tested birds' responses to the predator specifically during the breeding season, when delay in attending the nest either during incubation or during feeding young might have a strong negative effect on current reproduction. We do not know if chickadees from high elevation show this response specifically during breeding or if they might also be more cautious during other non-breeding contexts, such as foraging. However, there are no differences in response to novelty (which is a form of risk-taking) between high and low elevation chickadees in a feeding context (Kozlovsky et al. 2014), suggesting that the difference in risk-taking may only apply to a breeding context. Nonetheless, even if high elevation chickadees showed less risk taking in the presence of the predator no matter the context, it would still mean that during breeding such extra caution could jeopardize their current nesting attempt. In addition, it might be expected that birds with better spatial learning ability should indeed be more cautious in the presence of the predator at all times compared to individuals with inferior learning ability because of their superior foraging success.

We do not know whether birds from high and low elevation had different amount of exposure to hawks or whether hawk density is significantly different between elevations, which might also potentially affect bird responses. Sharp-shinned hawks are fairly common at both elevations and so we do not think that this explanation is likely.

It is theoretically possible that birds at higher elevation might experience higher predation risk causing them to be more cautious (even though we have no evidence or even a reason to suspect such differences). If this were the case, such extra caution would still mean higher self-investment at a cost to the current reproduction because predator-related delays might result in negative effects to current reproduction and given that the probability of encountering a predator should be higher, selection should favor individuals taking more risks as they might have higher fitness, unless such extra caution is associated with better survival allowing for multiple reproductive events. However, higher predation risk would mean lower probability of survival, which should favor parental effort and more risk taking. Better survival, on the other hand, should be linked to better spatial memory and the ability to recover food caches critical during the winter.

Finally, chickadees at high elevation generally experience lower ambient temperature, as temperature varies predictably along an elevation gradient (Hopkins & Powell 2001; Shepson & Tinnes 2003; Cook 2012) and this is especially pronounced during the incubation stage. However, as in all examples described above, in this case chickadees at high elevation should enter the nest sooner and delays to resume incubation or to feed young should have stronger negative consequences compared to lower elevations. Yet our results were exactly the opposite of these predictions.

While we might have missed some other potential explanations, among the considered alternative hypotheses the ‘the enhanced cognition - reduced parental risk taking’ trade-off hypothesis appears most consistent with our results showing mixed parental investment strategy – investing more in producing larger clutches but less in properly caring for both eggs and young when such caring involves more risks to parents own survival. Obviously our data cannot provide a causal link between better spatial learning ability and reduced risk taking, but they do tentatively support a potential association between these traits. Whereas we did not test spatial memory or problem solving in specific birds used in this study, significant elevation-related differences in spatial memory and the hippocampus have been previously well established at our study locations and such differences also persisted in a uniform captive environment (Freas et al. 2012; Freas et al. 2013a). Differences between high and low elevations were especially striking in the total number of hippocampal neurons with no overlap between elevations (Freas et al. 2012). Differences in problem solving were also found in different groups of birds from the same elevation in a different year (Kozlovsky et al. 2015, Ch. 1). Therefore, even though we cannot conclude that changes in memory and/or problem solving ability directly affect reproductive decisions, the birds from a population associated with better spatial memory, larger hippocampi, significantly more hippocampal neurons and better problem solving performance did show reduced risk taking in the presence of an adult predator associated with reduced fledging success despite investing more in larger clutches. Even though our inferences are based on comparing just two elevations rather than multiple elevations, these are intriguing preliminary results that fall in line with previous studies (e.g. Cole et al. 2012) and, at least indirectly, support the cognition-parental investment trade-off hypothesis. Future studies are necessary to directly test this hypothesis and to demonstrate that selection on better learning abilities also produce less risk taking personality trait.

Acknowledgements

We would like to thank The Ecology, Evolution and Conservation Biology doctoral program at University of Nevada, Reno for funds for this project. All animal procedures were in accordance with the University of Nevada Reno Animal Care and Use Protocol (00576). VVP was supported by NSF (IOS-1351295).

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Table 1.

Breeding data and fledgling development indices in mountain chickadees from high and low elevations. All values are Least Square Means and Standard Errors produced by General Linear Models and n represents the number of nests. Brood size is measured at fledgling day 16. All statistical comparisons were made using the nest mean as an independent statistical unit. For all development variables (but not for within-nest CV's), clutch or brood size were used as a covariates.

Variable	High Elevation [mean± SE (n)]	Low Elevation [mean± SE (n)]	F, p
Clutch size	8.32±0.28 (21)	6.52±0.21 (33)	$F_{1,51}$=19.59, $p < 0.001$
First egg date	May 31±0.92 (21)	May 19±1.16(33)	$F_{1,52}$ = 69.15, $p < 0.001$
Brood size	6.41±0.73 (13)	5.63±0.42 (28)	$F_{1,38}$ = 0.62, p = 0.43
Fledging success (number of fledged young/clutch size)	0.48±0.01 (21)	0.70±0.07 (33)	$F_{1,53}$ = 4.33, p = 0.042
Body mass, g	12.62±0.20(13)	12.88±0.14(29)	$F_{1,39}$ = 1.12, p = 0.29
Body mass CV	0.05±0.008(13)	0.05±0.005(29)	$F_{1,40}$ = 0.05, p = 0.83
Primary length, mm	16.48±0.48(13)	16.76±0.32(29)	$F_{1,39}$ = 0.24, p = 0.63
Primary length CV	0.07 ± 0.02(13)	0.08 ± 0.01(29)	$F_{1,40}$ = 0.23, p = 0.63
Primary asymmetry, mm	0.40±0.06(13)	0.47±0.04(29)	$F_{1,39}$ = 0.98, p = 0.33
Primary asymmetry CV	0.94 ± 0.09(13)	0.92 ± 0.06(29)	$F_{1,40}$ = 0.04, p = 0.84
Tail length, mm	16.10±0.74(13)	17.47±0.49(29)	$F_{1,39}$ = 2.36, p = 0.13
Tail length CV	0.11 ± 0.02(13)	0.10 ± 0.01(29)	$F_{1,40}$ = 0.13, p = 0.72
Tail asymmetry, mm	0.79 ± 0.07(13)	0.69 ± 0.05(29)	$F_{1,39}$ = 1.32, p = 0.26
Tail asymmetry CV	0.82 ± 0.08(13)	0.71±0.05(29)	$F_{1,40}$ = 1.34, p = 0.25
Egg mass, g	1.29 ± 0.02(21)	1.26 ± 0.01(34)	$F_{1,52}$ = 2.83, p = 0.10
Egg mass CV	0.04 ± 0.04(21)	0.04±0.04(34)	$F_{1,53}$ = 0.09, p = 0.76
Incubation length, days	12.72±0.20(18)	13.03±0.16(30)	$F_{1,46}$ = 1.45, p = 0.23
Time in the nest, days	21.85±0.46(13)	21.29±0.32(28)	$F_{1,39}$ = 0.99, p = 0.32

Figures

Fig. 1. Mean latency to enter the nestbox in the presence of a hawk and a dove model during the incubation stage of the breeding cycle for chickadees from high and low elevation (closed circles - low elevation, open circles - high elevation). Error bars represent SEM.

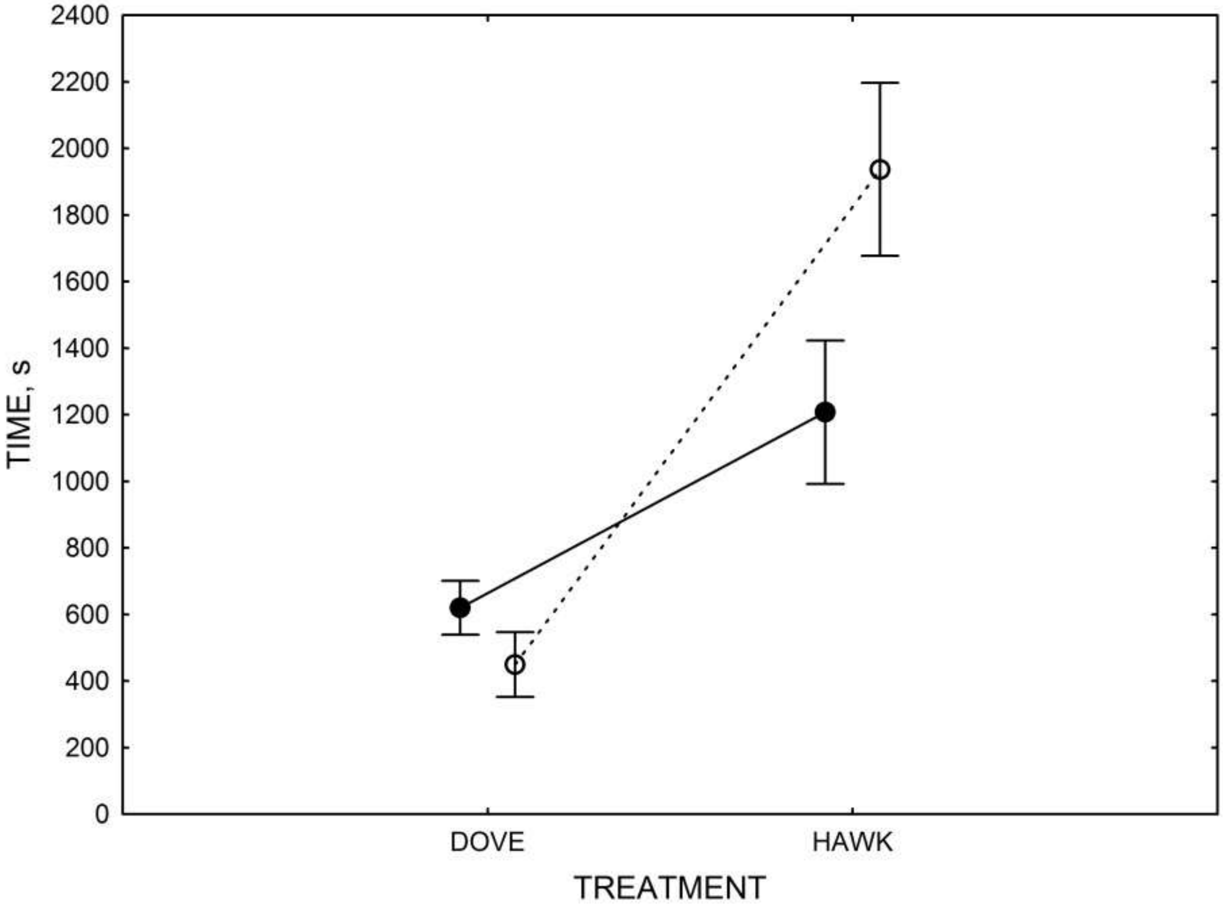


Fig. 2. Mean latency to enter the nestbox in the presence of a hawk and a dove model during the nestling feeding stage of the breeding cycle for birds from high and low elevation (closed circles - low elevation, open circles - high elevation birds). Error bars represent SEM.

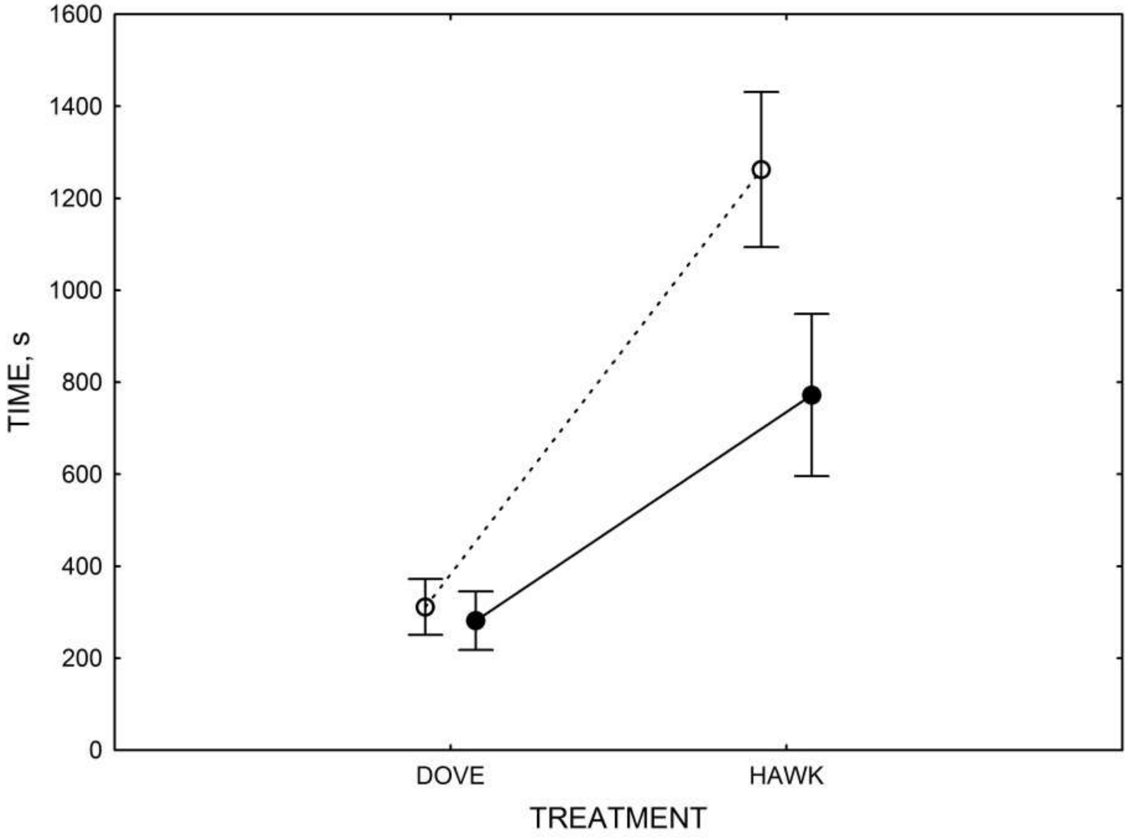
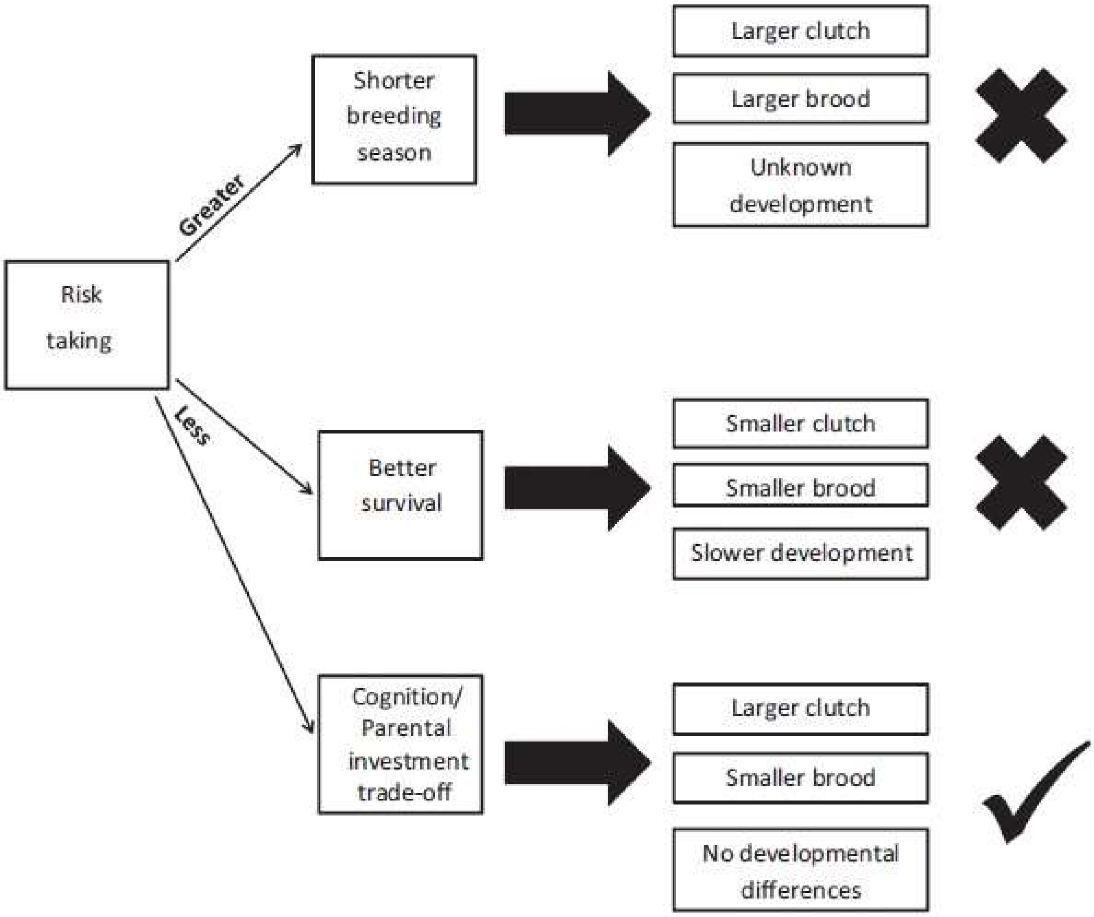


Fig. 3. Flow diagram of expected results for high elevation birds based on three potential hypotheses. An × means that our results are not in agreement with the expected results while a ✓ means our results are in agreement with expected results.



Ch. 3: What makes specialized food-caching mountain chickadees successful city slickers?

Dovid Y. Kozlovsky, Emily A. Weissgerber, Vladimir V. Pravosudov

Abstract

Anthropogenic environments are a dominant feature of the modern world, therefore, understanding which traits allow animals to succeed in these urban environments is especially important. Overall, generalist species are thought to be most successful in urban environments, with better general cognition and less neophobia as suggested critical traits. It is less clear, however, which traits would be favored in urban environments in highly specialized species. Here we compared highly specialized food-caching mountain chickadees living in an urban environment (Reno, Nevada, USA) with those living in their natural environment to investigate what makes this species successful in the city. Using a 'common garden' paradigm we found that urban mountain chickadees tended to explore a novel environment faster and moved more frequently, were better at novel problem solving, had better long-term spatial memory retention and had a larger telencephalon volume compared to forest chickadees. There were no significant differences between urban and forest chickadees in neophobia, food-caching rates, spatial memory acquisition, hippocampus volume, or the total number of hippocampal neurons. Our results partially support the idea that some traits associated with behavioral flexibility and innovation are associated with successful establishment in urban environments, but differences in long-term spatial memory retention suggest that even this trait specialized for food-caching may be advantageous. Our results highlight the

importance of environmental context, species biology, and temporal aspects of invasion in understanding how urban environments are associated with behavioral and cognitive phenotypes and suggest that there is likely no one suite of traits that makes urban animals successful.

Introduction

In a world subjected to ever-increasing anthropogenic activity many organisms must contend with urbanization. Identifying phenotypes that allow animals to be successful in urban environments is vital for understanding how animals will adapt, flexibly change or perish in the face of anthropogenic alterations. Many studies have addressed what makes animals successful in urban environments, focusing on traits such as ecology [1–3], physiology [4–10], commonness of the species in native habitat [1,11], brain size [2,3,12–15], cognition [16–21], and behavior [2,4,8,17,21–24]. Since urban environments are especially novel compared to natural environments and are characterized by an abundance of novel stimuli and food resources, certain behavioral and cognitive traits may be especially important for successful urban invasion and dwelling [20]. It has been suggested that individuals with behavioral traits that are often associated with bold behavioral profiles (e.g. fast novel environment exploration and reduced response to novel stimuli) will be most successful in initially invading urban environments [4,8,20], but over time these traits may be lost in more established urban populations [8]. Loss of traits associated with initial invasion in established populations may, in part, explain equivocal results of research on neophobia in urban populations (e.g. [21,22,25–27]). Furthermore, cognitive traits associated with behavioral flexibility and innovation (e.g.

large overall brain size and faster novel problem-solving) are also thought to increase success in urban environments [13,14,16,18,20,21,28]. Overall, most research has focused on generalists species, which are most common in the cities, yet it is unclear whether and how highly specialized species can be successful in urban environments.

Mountain chickadees (*Poecile gambeli*) are a resident, specialized food-caching species that inhabit the coniferous forests of western North America [29]. They have recently (within the last 25 years according to local bird watchers and historic ebird.org records) invaded the city of Reno, NV and appear to represent a breeding population (DYK and VVP personal obs.). Food-caching chickadees are pine seed specialists in the winter. They use cached food and recovery of cached food via spatial memory to survive [30–33], therefore, urban environments present unique challenges to specialized chickadees compared to generalist species [2,3]. The city of Reno has few native coniferous tree species (the preferred food and caching resource of mountain chickadees) and urban centers are characterized by an abundance of supplemental food sources from baited feeders. As a result, it is possible that urban-dwelling relaxes the demands for food-caching and accompanying spatial memory in urban chickadees, while increasing the need for generalist traits often thought to be important in urban environments.

Here we investigated whether mountain chickadees inhabiting urban environments (urban) differ from chickadees inhabiting their natural environment (forest) in behaviors that are thought to be necessary for initial urban invasion and successful urban dwelling. Specifically, we predicted that mountain chickadees from urban environments should explore a novel environment faster and be more active, should be less neophobic and better novel problem solvers, should cache less food and have worse spatial memory acquisition and retention associated with smaller hippocampus, but should have a larger telencephalon (a measure that is highly correlated to overall brain size in chickadees [31,34]) compared to forest chickadees.

Methods

Study subjects and capture sites

Starting in late October and concluding in early November 2014 thirteen mountain chickadees were captured from five locations around the city of Reno, NV (elevation range: ca. 1375-1525m; mean urbanization score = 1.68 based on the semi-automated scoring program detailed in [35]; see table S1) using both mistnets at established feeders and baited Potter traps. These locations included a park in the downtown core near the University of Nevada, Reno (4 birds) and from volunteers' backyards in South (2 birds), North (3 birds) and Southwest Reno (4 birds) neighborhoods. In early November 2014, twelve birds were captured using mistnets surrounding established feeders from low elevation coniferous forest (elevation range: ca. 1800-1900m; mean urbanization score = -2.55; table S1) at Sagehen Experimental Forest near Truckee, CA. Upon capture, birds were transported to our laboratory at University of Nevada, Reno where they were color banded, weighed and measured. Birds were housed individually in visually but not acoustically isolated cages (42 x 60 x 60 cm). Cage order was alternated so that birds from the same site were never in adjacent cages. Prior to any testing birds were habituated to laboratory conditions for at least two weeks with a white sheet covering their cages and an additional week without the sheet. Birds were fed a mixed diet of sunflower seeds, pine seeds, crushed peanuts, mealworms and insect pate and kept on a 9 h : 15 h light/dark schedule approximating Reno's shortest winter photoperiod. Two additional birds were brought in early January 2015. These chickadees were habituated and tested in an identical fashion to all previously captured birds. Overall, our samples had 9 males and 3 females from urban environment and 10 males and 3 females from forest environments. We were unable to identify the sex of 2 birds (these were excluded from any analyses where sex was significant).

Behavioral Experiments

Testing room

The first four behavioral experiments were conducted in the same testing room (218×373×263 cm) used in our laboratory's previous behavioral experiments [36,37]. Briefly, the testing room was adjacent to the bird housing rooms and birds were allowed into the testing room via trap doors and light manipulation [38]. Within the testing room there were four artificial trees (just trunks) each containing 20 perches with corresponding cache sites distributed equidistantly along the height of the tree (see S1 for testing room details). Each tree also had a top and a base, which the birds frequently landed on. The room also contained 12 perching blocks (9.01×4.5×4.0 cm; 6 on each of the shorter walls, evenly spaced) with one cache site each, making for a total of 100 intended perching and caching sites. Each cache site could be closed via a knotted white string that had to be removed to inspect the content of the site. Both of the longer walls had a one-way window through which all birds were observed.

Novel environment exploration

All birds were naïve to the testing room prior to the novel environment exploration experiment, which began in early December 2014 and followed the same procedures as in [37]. Testing began one hour after lights on and ran over the following two hours each morning until all birds were tested. Each bird was tested individually for a period of 30 minutes and the number of macro-substrates (i.e. the trees, the walls and the floor), the number of micro-substrates (i.e. the perches, planters and tree tops) used by the birds, and the number of flights were recorded by DYK observing through the one-way window. In addition to the intended perching substrates there were incidental perching substrates that were also counted toward micro-substrate totals. Such incidental substrates included door hinges, door stoppers, hinges for the trap door that allowed birds into the testing room and the alcove through which the bird entered the room. The number of new macro- and micro-substrates used by each individual were recorded cumulatively in 2-min blocks during the first ten minutes of the trial. This protocol took into account that the number of substrates were limited and expected to plateau as the trial continued. The number of flights (which included landing on the same substrates) were recorded cumulatively in 2-min blocks over the entire thirty minutes of the trial, as these measures are not limited by the number of substrates. Birds were returned to their cages via light manipulation after 30 minutes in the testing room.

Response to novelty

We tested response to novelty (e.g.neophobia) in each individual's home cage using an A-B-A design (same protocol used in [39,40]). Individuals were food deprived for one hour prior to lights off and two hours the next morning following lights on before testing.

All trials were video recorded. In the pre- and post-trial a familiar white feeder was placed on the floor of the home cage baited with a waxworm. During the neophobia trial the white feeder was replaced by one of four randomly assigned colored feeders with spokes (lime green, orange, pale pink or dark pink; Fig. 2 in [40]). The three trials occurred over three consecutive days (one per day). Trials ended when an individual took the waxworm or after thirty minutes. Latency to touch the feeder, land on the feeder, and take the waxworm from the feeder was scored from videos by DYK.

Problem-solving

Problem-solving trials were conducted in the homecage using a waxworm-baited upside-down test tube plugged with a cotton ball and clipped to the front of each bird's cage following our previous protocols (Fig. 1 in [40]). Chickadees could see the waxworm, but in order to solve the problem the birds had to pull the cotton plug to let the waxworm drop to the cage floor where it could be retrieved. Problem-solving trials were conducted without food deprivation as the purpose of the experiment was to test how birds spontaneously solve a problem when faced with a highly valued food item. Each trial lasted 1 h and trials were conducted twice per day (one approx. one hour after lights on and one at approx.1400). All trials were separated by at least four hours. Ten trials were given to all birds and an additional ten trials were given to individuals that failed to solve the problem during the first ten trials. All trials were video recorded. DYK recorded latency to first interact with the apparatus, the trial in which a bird first solved the problem, and how long it took a bird to solve the problem on the first and second trial in which it was solved.

Caching rates

Caching experiment methods generally followed [36]. Prior to the actual caching experiment, birds were given three hour-long habituation periods (once every third day) in the testing room (excluding the half hour period during the novel environment exploration experiment). Birds were food deprived for one hour prior to lights off the previous day and for one hour the next morning prior to beginning the caching trials. Each chickadee was given four trials, each separated by three days. Daily trial order was randomly assigned using a random number generator. Chickadees were provided with pine seeds, crushed peanuts and sunflower seeds (with and without the shell) in two bowls on either side of the testing room. Chickadees were allowed to cache for twenty minutes during which DYK or EAW recorded what was eaten, what was cached and where an item was cached. Additionally, we recorded the number of false caches (when a chickadee had a food item in its beak and stuck its beak in a cache site but did not cache the item in that location) and the number of re-caches (when a chickadee cached a food item and then removed it and cached the item in a new cache location). All food was removed from cached sites and the floor between trials. Each chickadee was given four trials, each separated by three days. The number of caches was averaged over the four trials.

One-trial associative learning task

A one-trial associative spatial learning task was conducted in the testing room using similar methods to those in [36]. Birds were food deprived one hour before lights off and two hours after lights on the following morning, when trials began. A randomly chosen cache site (of 100 available sites) was baited with a waxworm and all cache sites were in the open position (knotted string not covering the cache). During the pre-trial, each bird was allowed into the testing chamber and the trial concluded when the bird pecked at the worm. The lights were immediately shut off by the experimenter (either DYK or EAW) and the chickadee was not allowed to eat the waxworm. Chickadees were returned to their cages for a 20 min retention interval, after which the birds were allowed back into the testing room with all cache sites closed by the knotted string. The trial lasted until a bird found the baited cache or thirty minutes elapsed. The number and location of each incorrect cache site opened was recorded. Only trials where a chickadee was successful in finding the waxworm were counted. Each chickadee received four trials (one every fourth day) each one with a unique cache site. For those birds that never found the waxworm, a fifth and final trial was conducted. Performance was averaged over all successful trials.

Repeated associative learning task

A repeated associative spatial learning task followed similar protocol as in [36]. Each bird had a randomly chosen unique cache site (never the same cache site used in the one-trial experiment), which was openly baited with a waxworm and remained constant throughout the experiment. Every chickadee was given three pre-trials where all cache sites were open and in these three trials a chickadee was allowed to find and eat the waxworm (all birds rapidly found and ate the waxworm). Each chickadee received these trials and the subsequent repeated association trials every other day. During the repeated associative learning trials, chickadees were allowed into the testing room with all cache locations closed and their unique cache site containing a waxworm. Trials lasted 25 min or until the chickadee opened the correct cache site and ate the waxworm. Once an individual found the worm they were given an additional 5 min to eat the worm undisturbed before being returned to their cage. DYK or EAW recorded all incorrect cache sites a bird opened until it found the rewarded site. If an individual did not find the reward in a 25 min trial that trial ended and was not considered its first trial (this occurred with two birds). Nine trials were conducted followed by a 10th long-term retention trial (that occurred seventeen days after the 9th trial).

Brain Histology

Following all behavioral experiments chickadees were anaesthetized with an overdose of Nembutal© and their brains were prepared for histological analysis using our laboratory's well-established protocols [36,41–43]. Chickadees had their brains removed following a transcardial perfusion with 10 minutes of 0.1 M phosphate-buffer solution, then 15 minutes of 4% paraformaldehyde phosphate-buffer solution. Following brain removal gonadal inspection of the abdominal cavity for each bird was conducted to determine sex. Brains were put through a series of post-fixation solutions starting with a 4% paraformaldehyde solution for a week then two sucrose solutions until the brains sank (15% and 30% sucrose, respectively). The brains were then flash frozen in dry ice and stored in a -80°C freezer. Brains were sectioned at 40 µm sections with a Leica c 3050s cryostat and every 4th section was mounted for Nissl staining. The remaining sections were placed in cryoprotectant and stored in a -80°C freezer. Every 12th Nissl stained section was used for estimating hippocampus volume and neuron numbers. Additionally, every 16th section was used for estimating telencephalon volume (a measure that is highly correlated to overall brain size in chickadees [31,34]) using Stereo Investigator software and a Leica microscope fitted with a camera and connected to a computer. DYK measured the telencephalon and hippocampus blind to the site of origin for all brains using a Cavalieri estimator with a grid size of 1200 mm and 200 mm, respectively, following our laboratory protocols [36,43]. The total number of neurons was estimated using the Optical Fractionator method with a 250mm grid, a 30x30 mm counting frame, a 5mm dissector height and a 1mm guard following our previous work [44]. Brain cells were classified as neurons based on the following features: 1) having 1 or 2 dark stained nucleoli, 2) containing nucleoplasm, and 3) having dendrites projecting from the neuron soma. The two brain hemispheres were measured independently and summed together for the overall estimate.

Statistical analysis

STATISTICA computational software was used for all analyses. All behavioral and brain data analyses used t-tests and repeatedmeasures General Linear Models (GLM) and GLMs without repeated measures. All GLMs initially included sex as a predictor, but sex was always removed if it was non-significant. Data that did not meet normality assumptions were log-transformed.

Results

Behavioral Experiments

Novel environment exploration

There was no significant difference between forest and urban birds in the number of substrates visited across the first ten minutes of exploring the testing room (Repeated-measures GLM, $F_{1,25} = 1.19$, $p = 0.29$; Fig. 1A) and the interaction between time interval and site was not significant ($F_{4,100} = 0.18$, $p = 0.95$). When analyzing the first two minutes of exploration there was a non-significant trend for urban chickadees to land on more novel substrates (t-test$t_{25} = 1.78$, $p = 0.09$; Fig. 1A). There was a significant difference between forest and urban birds in the number of flights (Repeated-measures GLM, $F_{1,25} = 13.02$, $p = 0.001$; Fig. 1B). The interaction between time block and site for the number of flights was also significant ($F_{14,350} = 9.59$, $p < 0.001$) with urban chickadees flying significantly more than forest chickadees from the 8 min time block through the end of the trials (Fisher's Least Significant Difference: all p's < 0.05).

Response to novelty

All chickadees responded to the novel treatment by taking significantly longer to approach the novel feeder compared to control trials (Repeated-measures GLM, $F_{8,184} = 20.19$, $p < 0.0001$), but there were no significant differences between urban and forest chickadees ($F_{1,23} = 0.004$, $p = 0.9$) and the interaction between within-subject (novelty vs. control) and site was not significant ($F_{8,184} = 0.04$, $p = 0.99$; Fig. 2).

Problem-solving

Both urban and forest chickadees interacted with the testing apparatus within the first two min of the first trial with no differences between the groups ($t_{23} = 0.12$, $p = 0.9$). Urban chickadees solved the novel problem for the first time in significantly fewer trials than forest chickadees (log-transformed; $t_{23} = 2.31$, $p = 0.03$; table 1). All forest and urban birds solved the problem a second time in just one trial.

Caching rates

No significant differences were found between forest and urban chickadees for the mean caching rates ($t_{25} = 1.19$, $p = 0.24$; table 1). However, urban chickadees false cached significantly more often than forest chickadees ($t_{25} = 3.01$, $p = 0.006$; table 1) and there was a non-significant trend for urban birds to re-cache more compared to forest birds ($t_{25} = 1.96$, p = 0.06l; table 1).

One-trial associative learning task

There were no significant differences between urban and forest chickadees in the mean number of errors during the one-trial associative learning task ($t_{19} = 1.32$, $p = 0.20$; table 1)

Repeated associative learning task

Chickadees from both urban and forest environments did not differ significantly in number of errors during the first four trials of the repeat associative learning task (Repeated-measures GLM, $F_{1,23} = 0.18$, $p = 0.67$; Fig. 3) and there was no significant interaction between trial and origin of birds ($F_{1,23} = 1.11$, $p = 0.35$). Chickadees did, however, significantly improve from one trial to the next ($F_{4,92} = 3.70$, $p = 0.0078$; Fig. 3). The origin of birds was not a significant predictor when memory performance was compared during the 9th and 10th (e.g. long term) trials ($F_{1,21} = 1.03$ $p = 0.32$), but there was a significant interaction between trial number and bird origin ($F_{1,21} = 4.88$, $p = 0.04$; Fig. 3). The number of errors during the 10th trial after the long-term retention interval was significantly lower for the urban birds ($F_{1,21} = 10.14$, $p = 0.004$) showing better memory retention. Planned comparisons additionally showed the number of errors for the urban birds was not significantly different between the 9th and 10th (long-term) trials ($p = 0.5$), while the number of errors for forest chickadees was significantly higher after the long-term retention interval (e.g. difference between trial 9 and 10; $p = 0.001$). There were no significant differences between the forest and the urban chickadees in number of errors during trial 9 preceding the long-term retention interval ($p = 0.6$), but forest chickadees inspected significantly more incorrect cache sites than urban chickadees during trial 10 following the long-term retention ($p = 0.01$).

Brain Morphology

Bird's origin was not a significant predictor for telencephalon volume (GLM, $F_{1,21} = 0.012$, $p = 0.91$), however there was a significant difference between males and females ($F_{1,21} = 7.60$, $p = 0.01$) and the interaction between bird's origin and sex was also statistically significant ($F_{1,21} = 6.70$, $p = 0.02$; Fig. 4). Including body mass in the model as a covariate did not change the results (birds origin: $F_{1,19} = 0.04$, $p = 0.85$; sex: $F_{1,19} = 5.88$, $p = 0.03$; sex*origin: $F_{1,19} = 6.72$, $p = 0.02$) and the effect of body mass was not significant ($F_{1,19} = 0.85$, $p = 0.37$). Urban males had significantly larger telencephalon volume than forest males ($p = 0.02$) and females from either environment (urban females: $p = 0.001$; forest females: $p = 0.05$), however, there were no significant differences between urban and forest females ($p = 0.14$), urban males and forest females ($p = 0.08$) or forest males and forest females ($p = 0.91$; Fig. 4). When just analyzing males the results remained highly significant, with urban males having significantly larger telencephalon volume than forest males ($t_{19} = 2.75$, $p = 0.01$). Due to much smaller sample sizes, females were not analyzed separately.

There were no significant differences between urban and forest chickadees in the hippocampus volume, either relative to telencephalon volume or just raw hippocampus volume ($F_{1,22} = 0.22$, $p = 0.64$ and $F_{1,23} = 0.88$, $p = 0.36$, respectively; table 1). Telencephalon volume, however, was a significant covariate ($F_{1,22} = 5.33$, $p = 0.03$). There were also no significant differences between urban and forest chickadees in total number of hippocampal neurons (with telencephalon volume as a covariate) or in hippocampal neuron density (with hippocampus volume as a covariate) ($F_{1,22} = 0.08$, $p = 0.78$ and $F_{1,22} = 0.079$, $p = 0.78$, respectively; table 1). Telencephalon volume was not a significant covariate for the total number of hippocampal neurons ($F_{1,22} = 3.35$, $p = 0.08$), but hippocampus volume was a highly significant covariate ($F_{1,22} = 72.75$, $p < 0.0001$).

Discussion

We found that specialized food-caching mountain chickadees inhabiting the urban environment of Reno, NV possessed several generalist behavioral and cognitive traits thought to increase success in urban environments, while also being better at some specialized cognitive traits. Urban chickadees had a slight tendency to explore a novel environment faster while also exhibiting higher movement rates, solved a novel problem faster, and had a larger telencephalon compared to forest chickadees, albeit the last result being specific only to males. Additionally, urban chickadees false cached more, tended to re-cache more and showed significantly better spatial memory retention compared to forest chickadees. On the other hand, there were no significant differences between urban and forest chickadees in neophobia, food-caching rates, spatial memory acquisition, hippocampus volume or the total number of hippocampal neurons.

Succeeding in highly novel urban environments is not trivial for animals adapted to their natural environment. Urban environments are substantially different compared to natural ones and are characterized by a tremendous amount of novel stimuli. As a result, it has been suggested that bold personality traits such as the ability to rapidly explore a novel environment and reduced neophobia are critical for successful invasion into urban environments [4,8,20]. Our results provided only partial support for this idea; urban chickadees had a slight tendency to be faster explorers, but we found no evidence that they were less neophobic. Neophobia and exploration have been suggested to form a behavioral syndrome [4,45], but our results combined with our previous data comparing chickadees from two elevation extremes [37,40] suggest that these two traits might be independent, at least in mountain chickadees. Interestingly, it has been suggested that boldness and aggression are also independent in urban song sparrows [24], suggesting that behavioral syndromes may not be stable across different environments.

Our results showing no reduced neophobia in urban environment are not unique as previous studies have yielded equivocal results [21,22,25–27]. One possible reason for such inconsistency might be because different studies tested animals at different stages of invasion and establishment in urban environments. It has been suggested that faster exploration and reduced neophobia may only be beneficial during the early stages of invading an urban environments, but may be lost as animals become established [8] and learn that some of the novel stimuli may be associated with increased risk, which may favor an increase in neophobia. This idea, however, does not seem to be consistent with our data, as mountain chickadees appear to be relatively new invaders to the city of Reno (according to local bird watchers and historic ebird.org data), so it is unlikely that these traits have changed over such a short period of time. However, it remains unclear which traits may represent necessary traits for initial invasion and which traits can be expected to change as a result of establishment, especially within the confines of the current study. Population-level, longitudinal experiments at different stages of invasion may provide valuable data on this hypothesis and the temporal scale of such phenotypic changes. Another plausible explanation for these equivocal results is that these traits are highly dependent on the specific species ecology and life history as well as the specific urban environment in question. Studies addressing sister taxa with unique ecology or life history and studies of intraspecific populations inhabiting different urban environments are necessary to address these possibilities.

Once initial invasion has occurred animals must contend with unique challenges presented by urban environments. Generalist species have been suggested to be more likely to succeed in urban environments [2,3]. Increased behavioral flexibility, enhanced innovation (as measured by problem-solving; *sensu* [46,47]) and a larger brain (associated with the aforementioned traits; [48]) are thought to benefit generalists by allowing them to be flexible in the face of new challenges and by allowing access to otherwise inaccessible resources in novel urban environments [16,18,20,21,49,50]. Indeed, in our study, we found that urban chickadees were better at novel problem-solving and had larger brains (e.g. telencephalon volume) than forest chickadees. The fact that all birds that solved the problem the first time, regardless of bird's origin, solved the novel problem a second time in just one trial clearly demonstrates that learning did occur.

Interestingly, we found that larger brain size in urban chickadees was only evident in males, but not females. It is not clear why females might have smaller brains, however, our sample sizes for males were much larger than that for females (19 males and 6 females, with only 3 females per group) and such small sample sizes for females may potentially provide spurious results.

Mountain chickadees are thought to rely heavily on food caches and on spatial memory used to recover those caches to survive [30–33]. Considering the abundance of supplemental food and the lack of native pine seeds in the city of Reno, we expected urban chickadees to have reduced caching rates and worse spatial memory acquisition and retention. In contrast, we found no significant difference between urban and forest chickadees in caching rates and spatial memory acquisition. While it remains possible that urban chickadees rely on their caches and spatial memory as much as forest chickadees other explanations are also plausible. First, it is possible that the invasion of Reno by chickadees was so recent that caching and spatial memory have yet to be altered by the demands of Reno's urban environment and that chickadees that moved to Reno were not inferior in food caching propensity and spatial memory ability. Second, there are likely no disadvantages to caching in an urban environment and if birds moving to the city are not different than forest birds in these abilities, there may not be strong selection against food caching and spatial memory.

Most surprisingly and in contrast to our expectations, urban mountain chickadees showed better spatial memory retention compared to their forest counterparts.

Interestingly, chickadees from harsher, high elevations also exhibited better memory retention compared to the lower elevation chickadees [36], like those used for this study. These findings suggest that urban conditions may be harsh in some respects or that longer memory retention in urban and harsh environments may be favored by different demands. Considering that supplemental food at baited feeders is likely the main food source for urban chickadees during the winter and that such feeders are likely permanent but widely spaced, retaining memory of these distanced permanent feeders might be highly beneficial.

Urban chickadees false cached more and had a tendency to re-cache more than their forest counterparts. False and re-caching are strategies that may help prevent cache pilferage via the spread of misinformation [51]. While compared to natural chickadee environment, the number and density of chickadees appear lower in the city of Reno (DYK and VVP personal obs.), it is likely that the number of other potential species competitors for caches is higher in the city, especially during the crucial winter months and therefore, urban chickadees might engage in more cache protection.

Overall, urban chickadees appear to be more similar to chickadees from harsher, higher elevations in some traits while more similar to the lower elevation chickadees that surround the city in other traits. Urban and high elevation chickadees are both faster novel problem-solvers ([40] and this study) and have longer spatial memory retention compared to low elevation chickadees. On the other hand, urban chickadees had a (non-significant) tendency to explore more during the first 2 minutes of novel environment exploration and had significantly higher movement activity in the novel environment compared to low elevation chickadees, which, in turn are also faster explorers than high elevation chickadees [37]. At the same time, urban chickadees were similar to low elevation chickadees in food caching propensity, spatial memory acquisition and hippocampal morphology, while chickadees from harsher, high elevations cache significantly more, have better spatial memory acquisition and a larger hippocampus [36]. The similarities and differences between these three environments highlight the need to understand specific environmental-mediated abiotic and biotic factors associated with each of these traits and could be a fruitful avenue for future work.

It is important to note that the city of Reno is at a lower elevation than our low elevation forest sites. The difference between the highest point in Reno and the lowest point in the forest is ca. 275m. Due to Reno's geography it was impossible to choose urban sites that precisely matched the elevation of forest sites and therefore, it remains feasible that elevation-related factors might be contributing to some of our results. There are several reasons, however, that this is unlikely. For one, our previous study on elevation-related differences in traits involved in food-caching [36] found marginal to no differences in hippocampus volume, the total number of hippocampal neurons, or hippocampal neurogenesis rates between chickadees from ca.1200m elevation (lower than any urban site used for this study) and chickadees inhabiting the low elevation forest site used for this study (1800-1900m). Most dramatic and significant differences were found between birds from 1800-1900m and 2400m in elevation. This makes sense as it is typically thought that major climate-related elevation shifts (e.g. snow storms, lower temperature) occur above ca. 2100m in elevation. If elevation was mediating our results, it would be expected that all cache-related and cognitive traits for urban chickadees (caching, spatial memory, problem-solving and brain morphology) would be significantly lower than those of forest chickadees. Instead, we either found more similarities between the urban and high elevation chickadees or no significant differences. Strikingly, urban chickadees had larger brain size while no such differences were detected between forest chickadees from all elevations [36,40]. Low elevation chickadees explore significantly more in a novel environment compared to high elevation chickadees, however the urban chickadees did not explore significantly more than forest chickadees (albeit there was a slight trend). Elevation effects are only consistent with the results for flight activity in a novel environment, however for the reasons stated above it seems more likely that urban effects are responsible for these data.

These data also pose the question of whether urban environments are 'harsh environments' as previously suggested (e.g. [13]). While there is little doubt that urban environments are novel and likely less predictable than forest habitats [13], the abundance of stable supplemental food resources suggests that at least in some regards urban environments may be less harsh than natural habitats, especially during the winter. Caution should be taken when qualitatively labelling an environment harsh and careful operationalization of the term 'harsh' is necessary. Indeed, it may be detrimental to lump novelty with unpredictability and harshness when addressing how environments might favor different behavioral and cognitive phenotypes.

In the current study, urban and forest chickadees were brought into a 'common garden' laboratory setting to test for potential differences independent of immediate environmental conditions. Nonetheless, such design does not allow us to unambiguously determine whether any detected differences are due to potential differences in development, previous environmentally-induced behavioral plasticity or produced by natural selection. Variation in at least some of these traits have been suggested to be due to behavioral plasticity (e.g. problem solving; [52,53]), while variation in other traits have been hypothesized to be produced by selection (e.g. caching propensity and spatial memory: [30,32]; novel environment exploration: [54]; neophobia: [22]).

Overall, our results show that highly specialized species can be successful in invading novel urban environments and suggest that such success might be associated with some generalist traits (e.g. flexibility and innovation, larger brain size) and some specialists traits (spatial memory retention). Combined with our findings of no differences between urban and forest birds in other traits thought to be critical for novel environments, our results suggest that there might not be a single set of behavioral and cognitive traits that predict success in novel environments. Instead, such traits might vary depending on species natural history and specific novel environment context.

Ethics

All institutional, state, and federal guidelines for animal care and use were followed.

Data accessibility

All data are included in the electronic supplemental material.

Competing Interest

The authors declare no competing interests

Author's Contributions

DYK and VVP conceived of the study and analyzed the data. DYK collected all birds. DYK and EAW conducted all behavioral experiments. DYK conducted all brain analyses. All three authors wrote the manuscript.

Acknowledgements

We would like to thank Isabella Houston and Keri Thacker for help with animal husbandry and Chris Feldman, Zachary Ormsby, Jennifer Rippert and the staff of Rancho San Rafael for allowing us to trap and take chickadees. We thank Carrie Branch, Maria Tello Ramos and Angela Pitera for constructive criticisms. DYK and VVP were supported by the National Science Foundation (IOS1351295) grant awarded to VVP. The study was also supported by a research grant from the Animal Behavior Society to DYK.

Funding

DYK and VVP were supported by the National Science Foundation (IOS1351295) grant awarded to VVP. The study was also supported by a research grant from the Animal Behavior Society to DYK.

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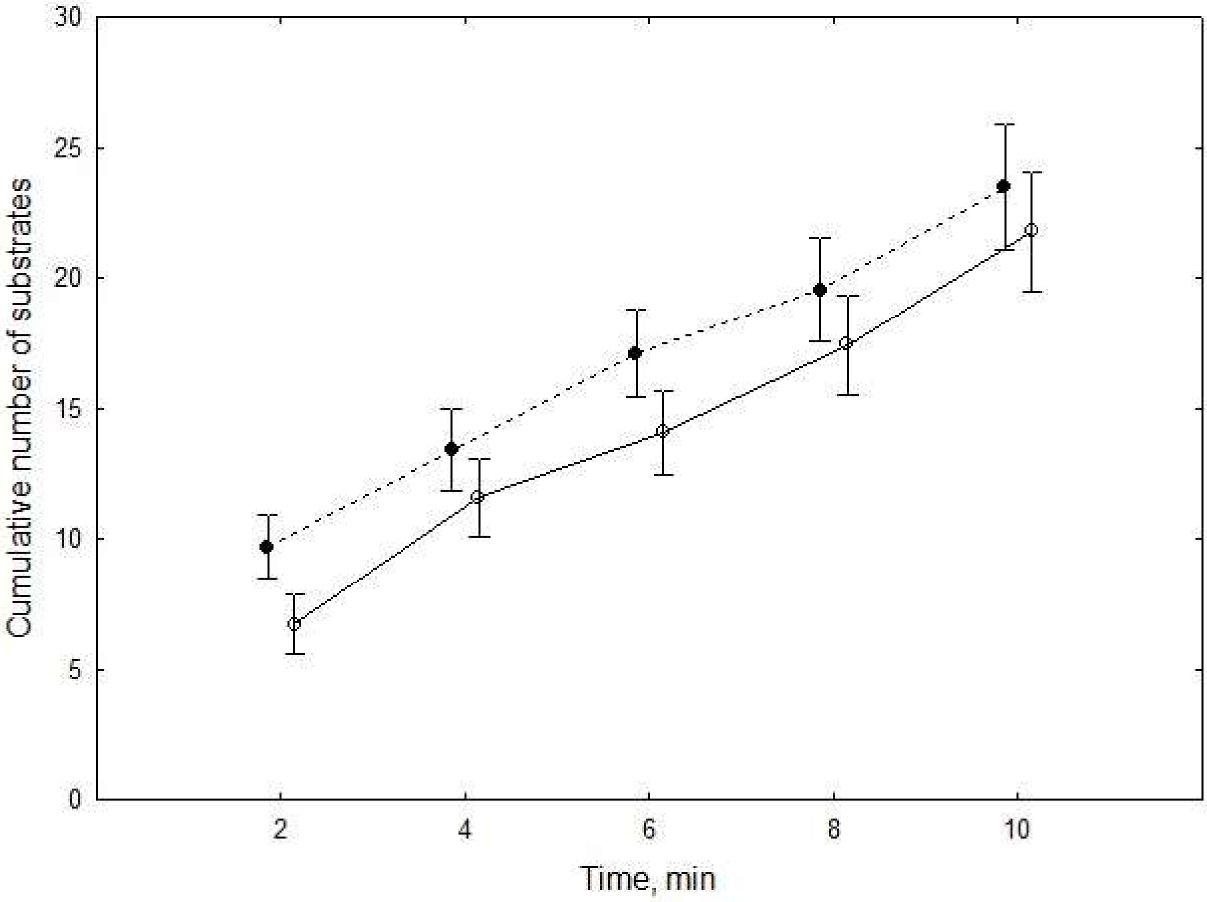
Table 1. Means, standard deviations (SD) and 95% confidence intervals (95% CI).

	Urban		**Forest**	
	Mean ± SD	**95% CI**	**Mean ± SD**	**95% CI**
Problem-solving (mean number of trials)	6.08 ± 7.50	1.32 -10.85	13.23 ± 8.15	8.31 - 18.15
Caching rates	13.63 ± 6.39	9.77 - 17.49	9.58 ± 10.58	3.47 - 15.69
False caching rates	1.23 ± 0.90	0.684 - 1.78	0.434 ± 0.407	0.199 - 0.670
Re-caching rates	1.04 ± 1.23	0.296 - 1.78	0.375 ± 0.447	0.117 - 0.633
One-trial Learning (mean number of errors)	5.65± 6.85	0.383 - 10.91	10.08 ± 8.15	4.90 - 15.26
Hippocampus Volume (mm^3)	16.28 ± 2.64	14.60 - 17.96	15.26 ± 2.75	13.60 - 16.93
Hippocampal Neurons	1467061 ± 280981.2	1288534 - 1645588	1387271 ± 303601.7	1203806 - 1570736

Figures

Fig. 1. Mean cumulative number of (A) new micro-substrates visited during 2-min blocks over a 10 min period and (B) flights (including return flights to the same substrates during 2-min blocks over a 30 min period for urban (closed circles, dashed line) and forest (open circles, solid line) chickadees. Error bars represent SE.

A.



B.

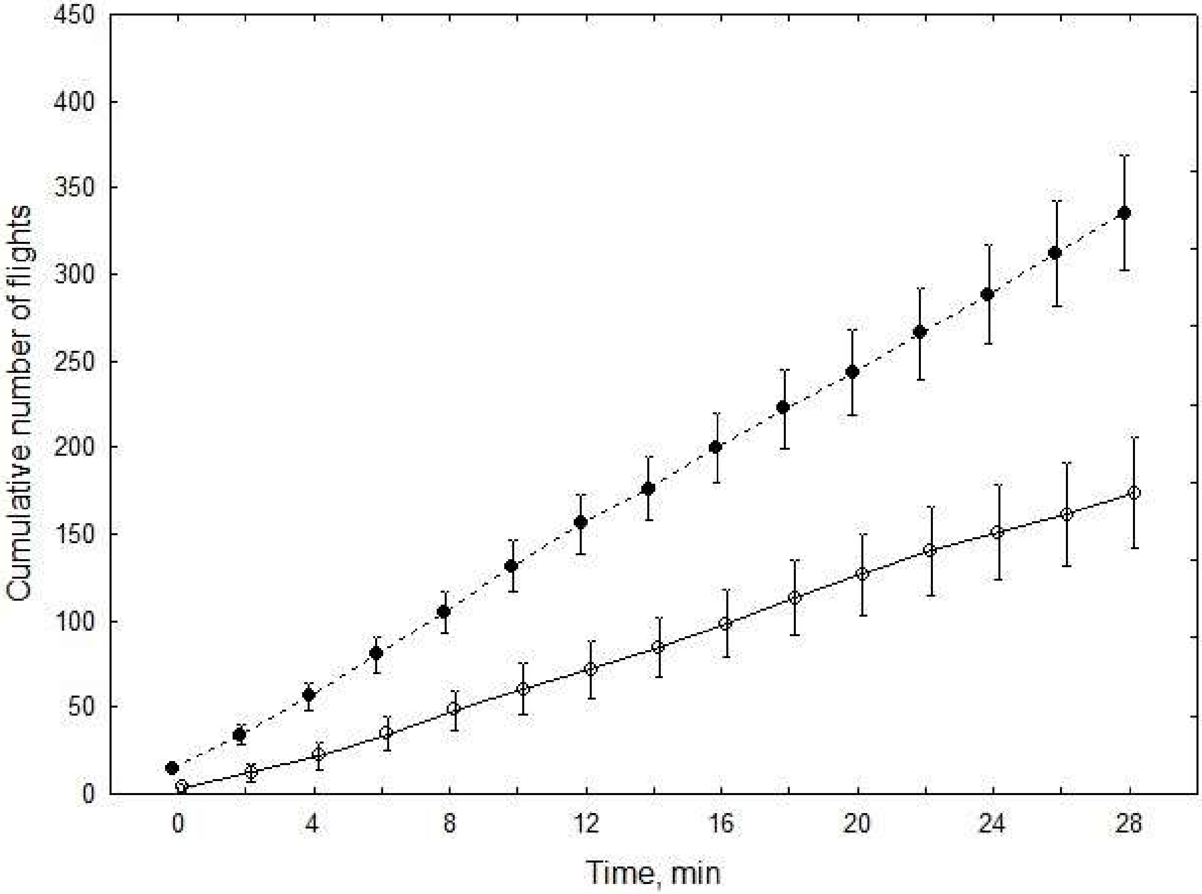

Cumulative number of flights
0
50
100
150
200
250
300
350
400
450
0
4
8
12
16
20
24
28
Time, min

Fig. 2. Latency to touch, land and take a waxworm during neophobia and control trials in urban (closed circles, dashed lines) and forest (open circles, solid lines) chickadees. Error bars represent SE.

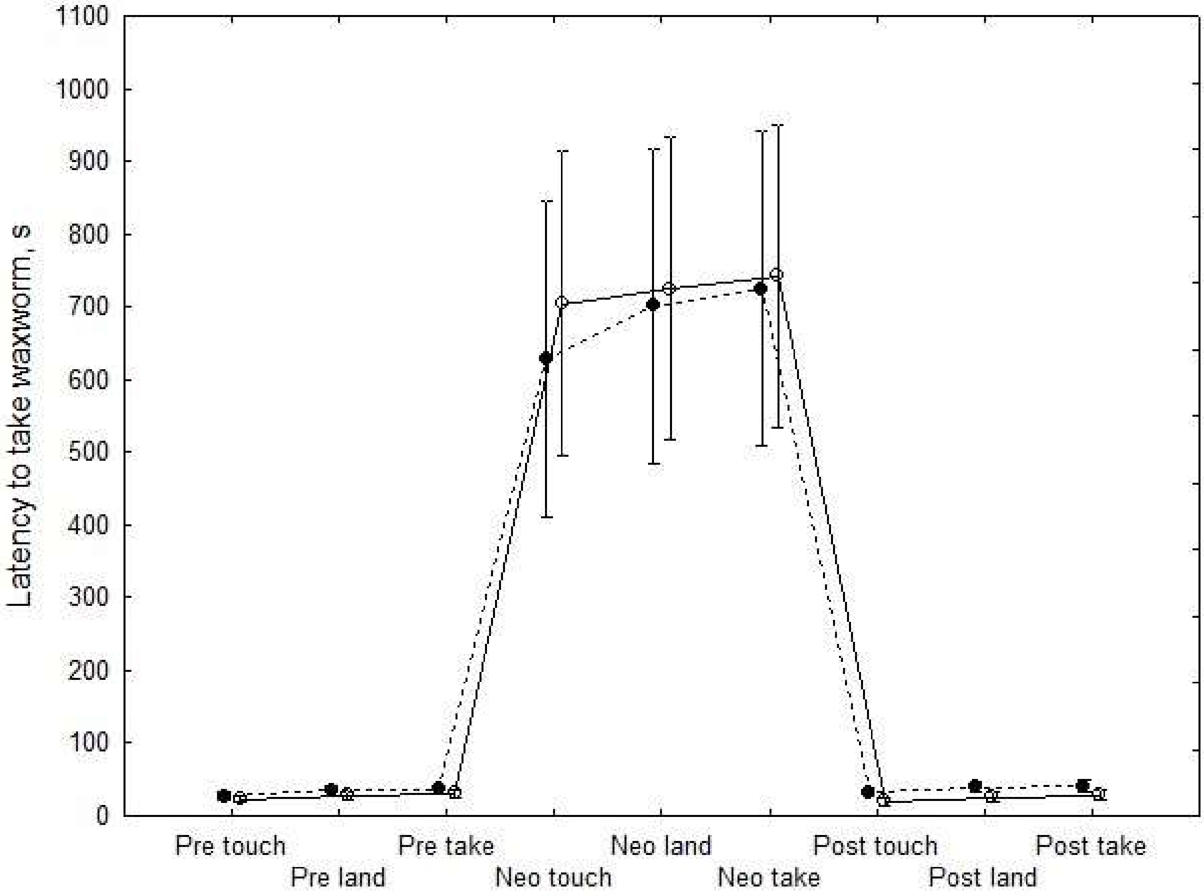


Fig. 3. Mean number of errors in a repeated-trial associative spatial learning task (including a long-term retention trial) for urban (closed circles, dashed lines) and forest chickadees (open circles, solid lines). Error bars represent SE.

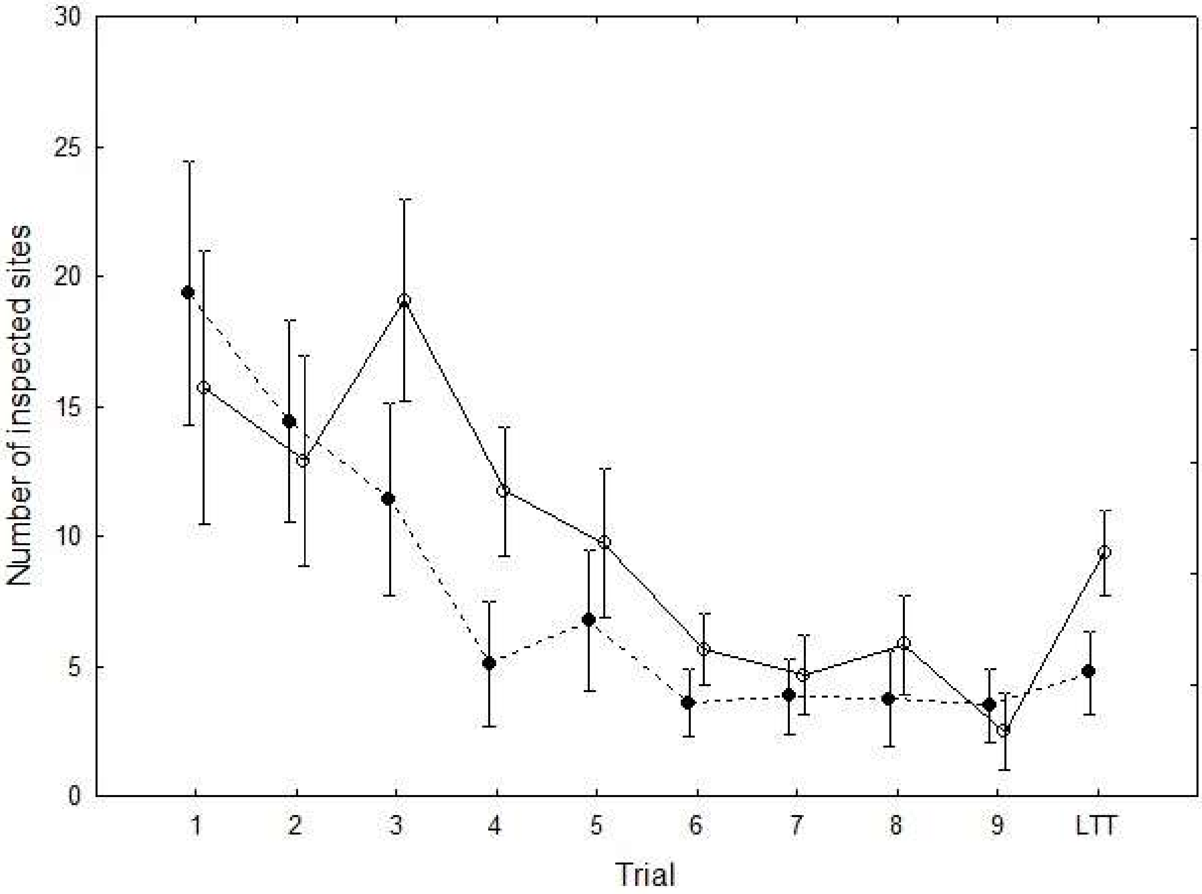
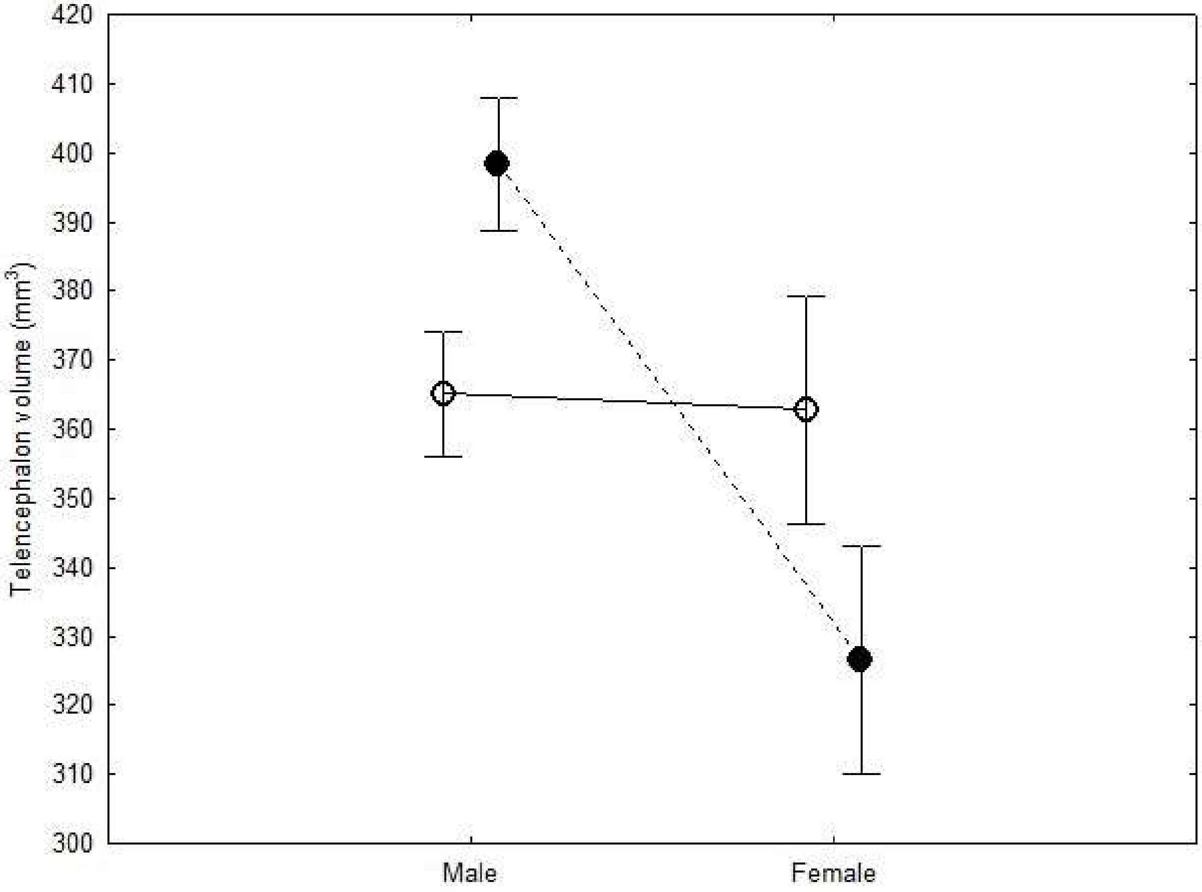


Fig. 4. Telencephalon volume for male and female chickadees in urban (closed circles, dashed lines) and forest (open circles, solid lines) habitats.



Supplemental Materials

Materials and Methods

Testing room

The first four behavioral experiments were conducted in the same testing room (218×373×263 cm) used in our laboratory's previous behavioral experiments [38,37]. Briefly, the testing room was adjacent to the bird housing rooms and birds were allowed into the testing room via trap doors and light manipulation [42]. Within the testing room there were four artificial trees (just trunks) each containing 20 perches with corresponding cache sites distributed equidistantly along the height of the tree (see S1 for testing room details). Each tree also had a top and a base, which the birds frequently landed on. The room also contained 12 perching blocks (9.01×4.5×4.0 cm; 6 on each of the shorter walls, evenly spaced) with one cache site each, making for a total of 100 intended perching and caching sites. Each cache site could be closed via a knotted white string that had to be removed to inspect the content of the site. Both of the longer walls had a one-way window through which all birds were observed.

Novel environment exploration

All birds were naïve to the testing room prior to the novel environment exploration experiment, which began in early December 2014 and followed the same procedures as in [37]. Testing began one hour after lights on and ran over the following two hours each morning until all birds were tested. Each bird was tested individually for a period of 30 minutes and the number of macro-substrates (i.e. the trees, the walls and the floor), the number of micro-substrates (i.e. the perches, planters and tree tops) used by the birds, and the number of flights were recorded by DYK observing through the one-way window. In addition to the intended perching substrates there were incidental perching substrates that were also counted toward micro-substrate totals. Such incidental substrates included door hinges, door stoppers, hinges for the trap door that allowed birds into the testing room and the alcove through which the bird entered the room. The number of new macro- and micro-substrates used by each individual were recorded cumulatively in 2-min blocks during the first ten minutes of the trial. This protocol took into account that the number of substrates were limited and expected to plateau as the trial continued. The number of flights (which included landing on the same substrates) were recorded cumulatively in 2-min blocks over the entire thirty minutes of the trial, as these measures are not limited by the number of substrates. Birds were returned to their cages via light manipulation after 30 minutes in the testing room.

Response to novelty

We tested response to novelty (e.g.neophobia) in each individual's home cage using an A-B-A design (same protocol used in [39,36]). Individuals were food deprived for one hour prior to lights off and two hours the next morning following lights on before testing. All trials were video recorded. In the pre- and post-trial a familiar white feeder was placed on the floor of the home cage baited with a waxworm. During the neophobia trial the white feeder was replaced by one of four randomly assigned colored feeders with spokes (lime green, orange, pale pink or dark pink; Fig. 2 in [36]). The three trials occurred over three consecutive days (one per day). Trials ended when an individual took the waxworm or after thirty minutes. Latency to touch the feeder, land on the feeder, and take the waxworm from the feeder was scored from videos by DYK.

Problem-solving

Problem-solving trials were conducted in the homecage using a waxworm-baited upside-down test tube plugged with a cotton ball and clipped to the front of each bird's cage following our previous protocols (Fig. 1 in [36]). Chickadees could see the waxworm, but in order to solve the problem the birds had to pull the cotton plug to let the waxworm drop to the cage floor where it could be retrieved. Problem-solving trials were conducted without food deprivation as the purpose of the experiment was to test how birds spontaneously solve a problem when faced with a highly valued food item. Each trial lasted 1 h and trials were conducted twice per day (one approx. one hour after lights on and one at approx.1400). All trials were separated by at least four hours. Ten trials were given to all birds and an additional ten trials were given to individuals that failed to solve the problem during the first ten trials. All trials were video recorded. DYK recorded latency to first interact with the apparatus, the trial in which a bird first solved the problem, and how long it took a bird to solve the problem on the first and second trial in which it was solved.

Caching rates

Caching experiment methods generally followed [38]. Prior to the actual caching experiment, birds were given three hour-long habituation periods (once every third day) in the testing room (excluding the half hour period during the novel environment exploration experiment). Birds were food deprived for one hour prior to lights off the previous day and for one hour the next morning prior to beginning the caching trials. Each chickadee was given four trials, each separated by three days. Daily trial order was randomly assigned using a random number generator. Chickadees were provided with pine seeds, crushed peanuts and sunflower seeds (with and without the shell) in two bowls on either side of the testing room. Chickadees were allowed to cache for twenty minutes during which DYK or EAW recorded what was eaten, what was cached and where an item was cached. Additionally, we recorded the number of false caches (when a chickadee had a food item in its beak and stuck its beak in a cache site but did not cache the item in that location) and the number of re-caches (when a chickadee cached a food item and then removed it and cached the item in a new cache location). All food was removed from cached sites and the floor between trials. Each chickadee was given four trials, each separated by three days. The number of caches was averaged over the four trials.

One-trial associative learning task

A one-trial associative spatial learning task was conducted in the testing room using similar methods to those in [38]. Birds were food deprived one hour before lights off and two hours after lights on the following morning, when trials began. A randomly chosen cache site (of 100 available sites) was baited with a waxworm and all cache sites were in the open position (knotted string not covering the cache). During the pre-trial, each bird was allowed into the testing chamber and the trial concluded when the bird pecked at the worm. The lights were immediately shut off by the experimenter (either DYK or EAW) and the chickadee was not allowed to eat the waxworm. Chickadees were returned to their cages for a 20 min retention interval, after which the birds were allowed back into the testing room with all cache sites closed by the knotted string. The trial lasted until a bird found the baited cache or thirty minutes elapsed. The number and location of each incorrect cache site opened was recorded. Only trials where a chickadee was successful in finding the waxworm were counted. Each chickadee received four trials (one every fourth day) each one with a unique cache site. For those birds that never found the waxworm, a fifth and final trial was conducted. Performance was averaged over all successful trials.

Repeated associative learning task

A repeated associative spatial learning task followed similar protocol as in [38]. Each bird had a randomly chosen unique cache site (never the same cache site used in the one-trial experiment), which was openly baited with a waxworm and remained constant throughout the experiment. Every chickadee was given three pre-trials where all cache sites were open and in these three trials a chickadee was allowed to find and eat the waxworm (all birds rapidly found and ate the waxworm). Each chickadee received these trials and the subsequent repeated association trials every other day. During the repeated associative learning trials, chickadees were allowed into the testing room with all cache locations closed and their unique cache site containing a waxworm. Trials lasted 25 min or until the chickadee opened the correct cache site and ate the waxworm. Once an individual found the worm they were given an additional 5 min to eat the worm undisturbed before being returned to their cage. DYK or EAW recorded all incorrect cache sites a bird opened until it found the rewarded site. If an individual did not find the reward in a 25 min trial that trial ended and was not considered its first trial (this occurred with two birds). Nine trials were conducted followed by a 10th long-term retention trial (that occurred seventeen days after the 9th trial).

Brain Histology

Following all behavioral experiments chickadees were anaesthetized with an overdose of Nembutal© and their brains were prepared for histological analysis using our laboratory's well-established protocols [38,43,40,41]. Chickadees had their brains removed following a transcardial perfusion with 10 minutes of 0.1 M phosphate-buffer solution, then 15 minutes of 4% paraformaldehyde phosphate-buffer solution. Following brain removal gonadal inspection of the abdominal cavity for each bird was conducted to determine sex. Brains were put through a series of post-fixation solutions starting with a 4% paraformaldehyde solution for a week then two sucrose solutions until the brains sank (15% and 30% sucrose, respectively). The brains were then flash frozen in dry ice and stored in a -80°C freezer. Brains were sectioned at 40 μm sections with a Leica c 3050s cryostat and every 4th section was mounted for Nissl staining. The remaining sections were placed in cryoprotectant and stored in a -80°C freezer. Every 12th Nissl stained section was used for estimating hippocampus volume and neuron numbers. Additionally, every 16th section was used for estimating telencephalon volume (a measure that is highly correlated to overall brain size in chickadees [31,34]) using Stereo Investigator software and a Leica microscope fitted with a camera and connected to a computer. DYK measured the telencephalon and hippocampus blind to the site of origin for all brains using a Cavalieri estimator with a grid size of 1200 mm and 200 mm, respectively, following our laboratory protocols [38,41]. The total number of neurons was estimated using the Optical Fractionator method with a 250mm grid, a 30x30 mm counting frame, a 5mm dissector height and a 1mm guard following our previous work [44]. Brain cells were classified as neurons based on the following features: 1) having 1 or 2 dark stained nucleoli, 2) containing nucleoplasm, and 3) having dendrites projecting from the neuron soma. The two brain hemispheres were measured independently and summed together for the overall estimate.

Tables and Figures

Figure ESM1. Labelled picture of the testing room.

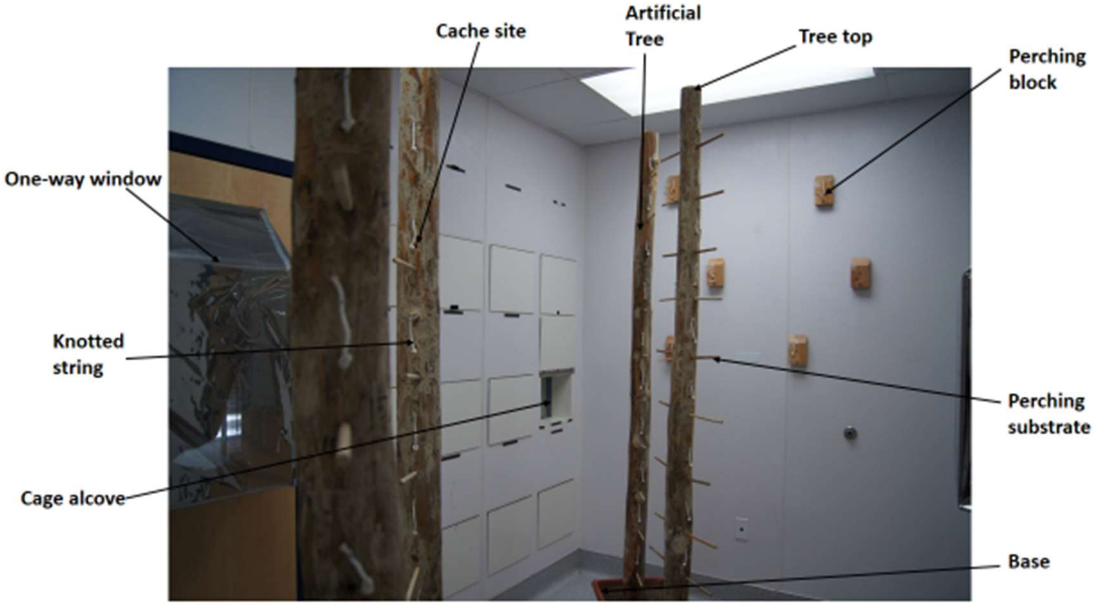


Figure ESM2. Mean cumulative number of new micro-substrates visited during 2-min blocks over a 10 min period for urban (closed circles, dashed lines) and forest (open circles, solid lines). Error bars represent SE.

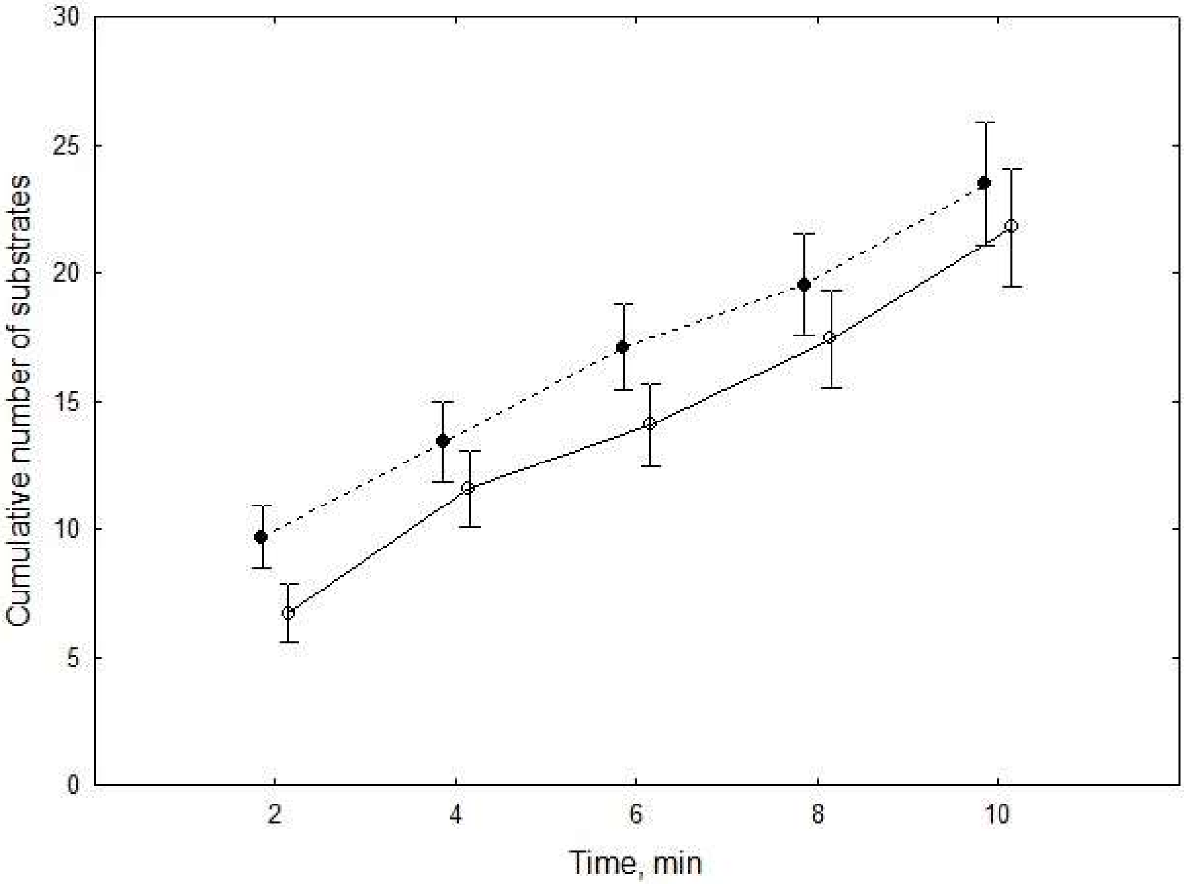


Figure ESM3. Latency to touch, land and take a waxworm during neophobia and control trials in urban (closed circles, dashed lines) and forest (open circles, solid lines) chickadees. Error bars represent SE.

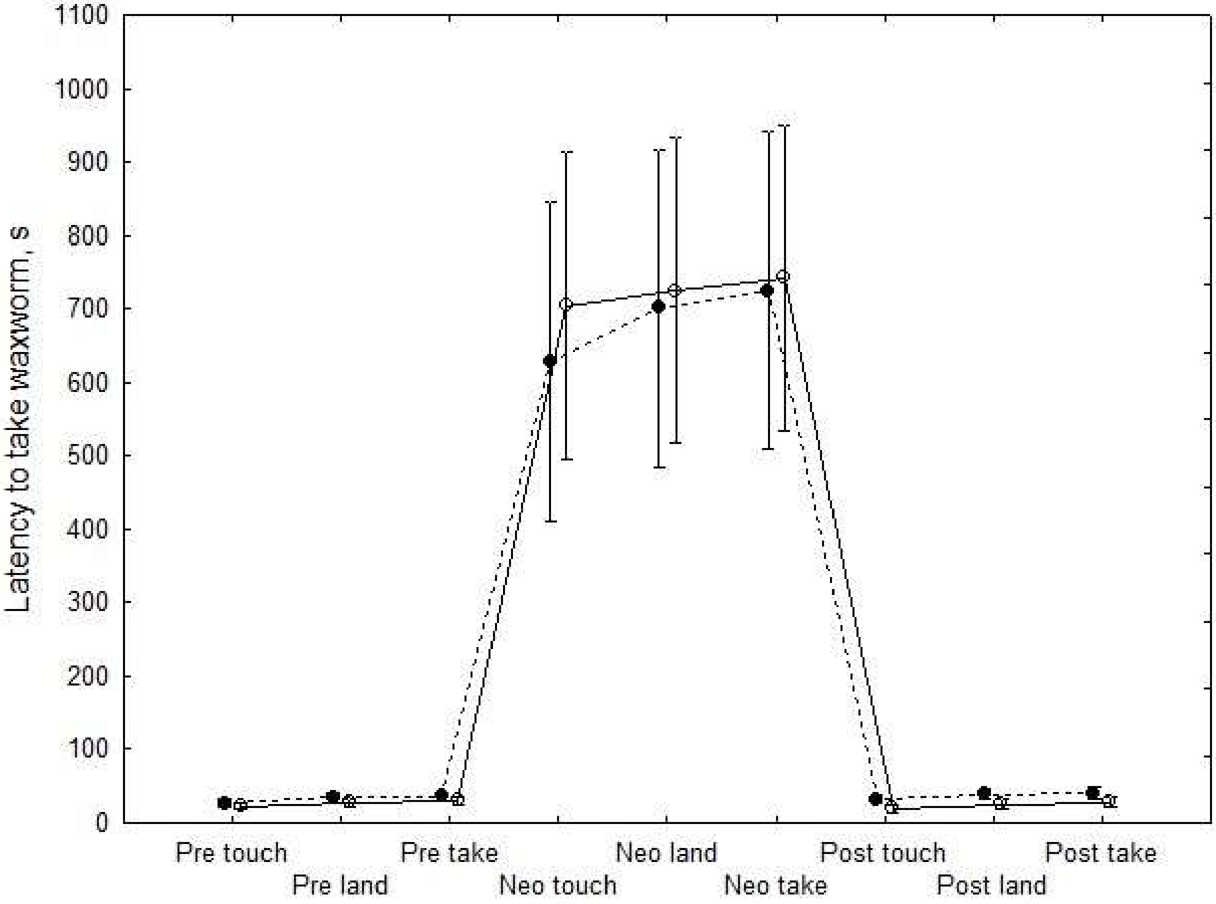


Table ESM1. The urbanization index used a semi-automated program designed by Seress et al. (2014). This index draws a 1 km x 1 km grid around a given location and breaks the grid into 10 m x 10 m cells. Within each cell the proportion of buildings and vegetation is calculated and the presence (a score of 1) and absence (a score of 0) of paved surfaces (R) is determined. Buildings (B) and Vegetation (V) can have values of 0 (percent cover is 0), 1 (percent cover is 0 > and ≤ 50%) and 2 (percent cover > 50%). Six summary statistics are calculate based on these values: the number of cells where B = 2 (>50%), number of cells where V = 2 (>50%), number of cells were paved surfaces are present (i.e. R = 1), mean building value (i.e. Avg. B; range = 0-2), mean vegetation value (i.e. Avg. V; range = 0-2) and an urbanization score (D; calculated as PC1 from a principal component analysis for the five aforementioned variables). For additional details see Seress et al. (2014).

At each location (5 urban and 3 forest) the program was trained by choosing 5 examples for each of the 3 land-cover characteristics (buildings, vegetation and paved surfaces). Table values represent the mean scores for the urban (range = 0.18 - 3.29) and the forest (all D's = -2.55).

Site	**B = 2**	**V = 2**	**R = 1**	**Avg. B**	**Avg. V**	**D**
Urban	52	35.25	71	1.34	1.3325	1.68
Forest	0	100	0	0	2	-2.55

Table ESM2. Means, standard deviations (SD) and 95% confidence intervals (95% CI).

	Urban		**Forest**	
	Mean ± SD	**95% CI**	**Mean ± SD**	**95% CI**
Problem-solving (mean number of trials)	6.08 ± 7.50	1.32 -10.85	13.23 ± 8.15	8.31 - 18.15
Caching rates	13.63 ± 6.39	9.77 - 17.49	9.58 ± 10.58	3.47 - 15.69
False caching rates	1.23 ± 0.90	0.684 - 1.78	0.434 ± 0.407	0.199 - 0.670
Re-caching rates	1.04 ± 1.23	0.296 - 1.78	0.375 ± 0.447	0.117 - 0.633
One-trial Learning (mean number of errors)	5.65± 6.85	0.383 - 10.91	10.08 ± 8.15	4.90 - 15.26
Hippocampus Volume (mm^3)	16.28 ± 2.64	14.60 - 17.96	15.26 ± 2.75	13.60 - 16.93
Hippocampal Neurons	1467061 ± 280981.2	1288534 - 1645588	1387271 ± 303601.7	1203806 - 1570736

Table ESM3. Regression analyses between elevation within the urban environment and response variables. + is statistically significant

	b*	SE of b*	t	df	p
Novel environment exploration	0.7	0.20	3.76	11	0.003$^+$
Flights in novel environment	0.4	0.28	1.40	11	0.2
Neophobia	0.2	0.31	0.81	10	0.4
Problem-solving (log-transformed)	0.50	0.27	1.85	10	0.09
Caching rates	0.020	0.30	0.066	11	0.9
False caching rates	-0.14	0.30	0.48	11	0.6
Re-caching rates	-0.26	0.29	0.88	11	0.4
One-trial learning	0.13	0.37	0.34	7	0.7
Long-term Retention	-0.2	0.31	0.66	10	0.5
Telencephalon Volume	0.43	0.28	1.52	10	0.16
Hippocampus Volume	0.2	0.31	0.65	10	0.5
Hippocampal Neurons	0.2	0.31	0.65	10	0.5

Conclusion

The results of my dissertation demonstrate variation for some cognitive and behavioral traits in different environments, while other traits were similar across environmental contexts. This suggests that environments that have different environmental factors are associated with some unique and other overlapping traits. In chapter 1, I found that mountain chickadees from high elevation had better problem-solving ability compared to birds from low elevation but no significant difference in response to novelty was detected. In chapter 2, I tested three hypotheses related to parental investment vs. self-investment when adults are faced with increased perceived risk of predation during reproduction and found that parents with better cognition (both spatial memory and problem-solving ability) at high elevation were less willing to take a risk to themselves even if that negatively impacted their reproductive opportunity compared to low elevation individuals. This suggests that there may exists a cognition and risk-taking trade-off. In chapter 3, I addressed what behavioral traits make specialized food-caching animals successful in an urban environment and the results suggested that many generalist behavioral traits as well as a couple specialist behavioral traits are associated with chickadee's success in the urban environment.

The results of this dissertation in combination with previous work on food-caching chickadees from different environments (Freas et al. 2012; Kozlovsky et al. 2014a, b; Roth et al. 2010; Roth & Pravosudov 2009) provide striking comparisons in cognitive and other behavioral traits between animals from environments that differ in climatic harshness and those that differ in anthropogenic activities. Some traits, such as response to novelty, were similar across short spatial scales (i.e. across urbanization and elevational gradients), yet were different across latitudes (Roth et al. 2010). This suggests that some other feature of latitudes besides climatic harshness may be driving this difference across such a large spatial scale. Other traits (e.g. novel problem-solving ability, long-term spatial memory), appear to be associated with success in both highly urbanized environments and harsh environments, but not in more mild environments. While still others (e.g. food-caching rates, spatial memory acquisition, hippocampus morphology) are statistically similar between animals from urbanized environments and more mild environments, but success at high latitudes (Roth et al. 2010; Roth & Pravosudov 2009) and high elevations (Freas et al. 2012) are associated with enhancement in those traits. For novel environment exploration, urbanized animals appear to be more active than animals from milder environments which are more active than animals from harsher environments. Interestingly, overall brain size appears to be uniquely associated with each environment, with chickadees from high latitudes having large brains then those from low latitudes, chickadees from the two elevations having similar brain sizes and there being a sex difference in associations of brain size with urban and natural environments.

While the resulting comparisons across different environments provide clues to what traits may be beneficial under different environmental conditions, the exact association between these traits and the given multitude of environmental factors remains poorly understood. Future laboratory ‘common garden’ experiments, tightly controlled field experiments, longitudinal studies, and broad-scale comparative studies should attempt to tease apart what specific environmental factors cause changes in specific behavioral and cognitive traits (as well as other phenotypes). Understanding this causal relationship will allow scientists to better identify and predict what species and populations are most likely to succeed and what traits these animals are likely to possess in environments that are changing naturally and anthropogenically across space and time.

Additional research is also needed to identify what traits are different across environmental gradients because of natural selection and what traits are different because of phenotypic plasticity. The difference is potentially important for many reasons. Firstly, the temporal dynamics of phenotypic changes are drastically different between the two mechanisms. Phenotypic plasticity occurs quickly within individuals, whereas the process of natural selection causes differences in the population norm of a given phenotype over generations (e.g. Chevin et al. 2013). These differences between plasticity and response to selection have potentially huge impacts on how organisms and populations can respond to rapid changes in the environment, as well as the ability of organisms or populations to establish in novel environments. A hypothetical example can be illustrated using an organism possessing a single trait with two forms. If one form of this trait is vital in natural environments and another is vital in urbanized environments and the difference between the two forms is a result of phenotypic plasticity, then most individuals that contend with the rapid onset of urbanization would quickly respond to this environmental perturbance and will therefore, have a high chance of successfully establishing in the new urban environment, resulting in a large and strong urban population. However, if these two forms differed because of natural selection then only those individuals that possess the genes for the urban-adapted behavior will be able to succeed and reproduce in the novel urban environment. Since the natural form is vital for the original environment, this urban form will likely be less common and therefore (at least initially) the new urban population will likely be small and may only become a well-established over successive generations. Obviously, this is a highly oversimplified example, however, it highlights a potential importance of understanding what traits are adaptive and what traits are phenotypically plastic. A second reason these differences are important is that phenotypic plasticity itself may affect an individual’s ability to survive and reproduce and therefore may impact what traits are selected and how individual’s may respond to selective forces (e.g. Grenier et al. 2016; Forsman 2015). A third reason is that certain environments (such as those that are predictably unpredictable or predictably variable) may be more often associated with phenotypically plastic traits than traits that are under strong selection, as drastic swings in environmental factors may relax selection on any one suite of rigid (non-plastic) traits which would therefore allow scientists to make better hypotheses about the mechanisms and types of traits associated with these different environments.

To conclude, spatial and temporal environmental heterogeneity are associated with differences in cognitive and other behavioral traits that have potentially important implications for an animal’s fitness. The impact of these behavioral differences depends on a multitude of factors such as, the specific environmental context, species ecology, and species life history. While environmental-associated differences in behavior, in general, and cognition in particular, are well studied both theoretically and empirically (including this dissertation), the causal relationship between environment, behavior, cognition, and fitness remain poorly understood.

Introduction and Conclusion Literature Cited

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