

University of Nevada, Reno

**Spatial Learning and Memory, Cognitive Flexibility, and
Rule-Learning in Food-Caching Mountain Chickadees (*Poecile gambeli*)
Across an Elevational Gradient**

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

by

Lauren Benedict Nguyen

Dr. Vladimir V. Pravosudov/Dissertation Advisor

May, 2024

© Copyright by Lauren Benedict Nguyen 2024

All Rights Reserved



THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

entitled

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

Advisor

Committee Member

Committee Member

Committee Member

Graduate School Representative

Markus Kemmelmeier, Ph.D., Dean
Graduate School

Abstract

How and why individuals differ in their cognitive abilities within and across species remain important and unresolved questions in behavioral and evolutionary ecology. Cognitive traits are involved in many critical behaviors, enabling individuals to use past experiences to inform future decisions or to reduce unpredictability in the environment. But most cognitive experiments have been conducted in laboratory conditions without a strong ecological or evolutionary context, and so do not address to what extent environmental factors influence cognitive traits and associated behaviors. Moreover, although many behaviors likely involve multiple cognitive traits, it is still unclear to what extent there may be tradeoffs between cognitive traits or between higher-order executive control functions. Executive control functions are associated with goal-oriented behaviors and may be highly advantageous, as they are involved with rule learning and using abstract, relational concepts; however, they remain highly controversial in nonhuman animals and have rarely been studied outside of a laboratory context. In this dissertation, I designed and conducted several spatial cognitive tasks to investigate individual variation in spatial learning and memory, cognitive flexibility, and rule-learning in wild, food-caching mountain chickadees (*Poecile gambeli*). I found that chickadees updated information about their environments more frequently in harsher, less predictable winter environments than in milder, more predictable ones. I showed that chickadees were capable of learning and using multiple different rules within a foraging context, and I demonstrated some of the first evidence for abstract-rule learning in nonhuman animals in the wild. Moreover, proactive interference associated with learned associations from

two cognitive tasks appeared to influence subsequent foraging decisions and varied with individual cognitive ability on two spatial tasks. Altogether, these results provide new insight into the cognitive ecology of mountain chickadees and the cognitive traits that may play a role in shaping information-related behaviors.

Dedication

To my Grandad, who taught me curiosity, and my Nana, who showed me how to explore the natural world. To my sister, who helped me find my resolve. To my family, who has supported me through every chapter, and to my husband, who has learned more about birds than he ever dreamed he would.

Table of Contents

Abstract	i
Dedication	iii
Table of Contents	iv
List of Tables	v
List of Figures	vi
Introduction.....	1
Chapter 1 Elevation-Related Differences in Annual Survival of Adult Food-Caching Mountain Chickadees Are Consistent with Natural Selection on Spatial Cognition.....	14
Chapter 2 Variation in Sampling Behavior is Associated with General Rather Than Local Environmental Conditions, Age and Cognitive Abilities	44
Chapter 3 Food-Caching Chickadees Do Not Exhibit Directional Bias When Learning a Spatial Task.....	109
Chapter 4 Learning Predictably Changing Spatial Patterns Across Days in a Food-Caching Bird	160
Chapter 5 Food-Caching Mountain Chickadees Can Learn Abstract Rules to Solve a Complex Spatial-Temporal Pattern.....	259
Conclusions.....	316

List of Tables

Table 3.1	151
Table 4.1	226
Table 4.2	227
Table 4.3	228
Table 4.4	229
Table 4.5	230
Table 4.6	231
Table 4.7	232

List of Figures

Figure 1.1	40
Figure 1.2	41
Figure 1.3	42
Figure 1.4	43
Figure 2.1	86
Figure 2.2	87
Figure 2.3	88
Figure 2.4	89
Figure 2.5	90
Figure 2.6	91
Figure 2.7	92
Figure 3.1	152
Figure 3.2	153
Figure 3.3	154
Figure 3.4	155
Figure 3.5	156
Figure 3.6	157
Figure 3.7	158
Figure 4.1	233
Figure 4.2	234
Figure 4.3	235

Figure 4.4	236
Figure 4.5	237
Figure 4.6	238
Figure 4.7	239
Figure 4.8	240
Figure 4.9	241
Figure 5.1	287
Figure 5.2	288
Figure 5.3	289
Figure 5.4	290

Introduction

Life is uncertain. Weather conditions change hourly, organisms behave according to their own unpredictable interests, and resources perish and replenish. This creates a constantly shifting information landscape in which an individual's certainty in the state of their surroundings progressively diminishes as information becomes outdated (Dall et al., 2005). The consequences of making decisions based on outdated information could range from mild, e.g., wasted energy foraging in a previously exploited patch, to disastrous, e.g., starvation as the result of poor anticipation of future resource distributions. As such, unpredictable environments should favor the evolution of behavioral strategies aimed at reducing environmental uncertainty and allowing better-informed decision-making.

Animals gather information, defined as a measure of the uncertainty of an outcome (Shannon-Weaver entropy, cited in Dall et al., 2005), to inform a wide variety of decisions. Females glean information on mate quality from various signals such as plumage, song quality, and courtship rituals (e.g., Candolin, 2003) while males gather information through signaling to ascertain the quality of other males and predict the outcome of competitive interactions (e.g., Garcia et al., 2019). Foragers guide their movements using socially and individually learned information on patch quality, patch location, and predator risk (Stephens, 2008). An individual that can remember this information might use it to predict the outcomes of reproductive success, competitive interactions, or foraging strategies, and therefore might have the potential to improve its fitness outcomes (Morand-Ferron et al., 2016). The neuronal processes that facilitate

these actions – the acquisition, retention, and usage of information – are collectively called cognition (Dukas, 2004).

A primary aim of cognitive ecology is to understand the ecological and evolutionary mechanisms that give rise to variation in cognitive traits (Hutchins, 2010; Pravosudov, 2022). Cognitive traits refer to the different ways these processes integrate to give rise to cognition-based behaviors, such as perception, memory, learning, attention, and decision-making (Dukas, 2004; Shettleworth, 2010). Like many physical traits, cognitive traits show substantial and heritable inter- and intraspecific variation in many taxa (Dukas, 2004; Morand-Ferron et al., 2016; Shettleworth, 2010; Sonnenberg et al., 2019) and may have a genetic basis (Branch et al., 2022). Variation in cognitive traits has also been linked to fitness outcomes (Croston et al., 2015; Morand-Ferron et al., 2016; Sonnenberg et al., 2019). For example, bumblebees with better performance on a color-based learning task also had higher foraging efficiency, which has been associated with better reproductive fitness for the colony as a whole (Raine & Chittka, 2008). Moreover, there is recent evidence that natural selection can act on a cognitive trait: food-caching birds with better spatial learning and memory ability, associated with better cache retrieval, were more likely to survive harsh winters than birds with worse spatial learning and memory (Sonnenberg et al., 2019). Yet despite these important advances in cognitive ecology, there are still few studies that show similar, direct evidence for selection on cognition (Morand-Ferron et al., 2016; Morand-Ferron & Quinn, 2015; Pravosudov, 2022).

There are several inherent challenges in studying cognition which might explain the dearth of direct evidence for selection on cognitive traits. Cognitive traits are highly plastic, sensitive to subtle variations in many different factors, and are deeply rooted in physical neural structures that may be poorly understood or difficult to study in the target taxa (Dukas, 2004; Pravosudov & Roth, 2013). Although carefully designed cognitive tasks have proven effective for studying cognition in live animals, performance typically depends on multiple cognitive and non-cognitive traits (e.g., motor abilities, motivational state) that can be difficult to distinguish (Morand-Ferron et al., 2016; Pravosudov, 2022). Moreover, many cognitive tasks are conducted with captive animals in laboratory conditions, often using paradigms that lack a strong ecological justification or elevate participants' stress responses (Pravosudov, 2022). While laboratory experiments provide controlled conditions and are undoubtedly valuable for studying mechanisms, these conditions limit insight into the ecological and evolutionary contexts necessary to understand natural selection (Morand-Ferron, 2017; Morand-Ferron et al., 2016; Pravosudov, 2022). Furthermore, correlations between fitness and cognitive traits may be masked by confounding variables or may be constrained by tradeoffs with other traits (Morand-Ferron & Quinn, 2015). Cognition may be energetically expensive and can direct resources away from other traits that may be necessary for behaviors like competition for resources or reproduction (Morand-Ferron & Quinn, 2015; Tello-Ramos et al., 2019). There may also be physical limitations that restrict investment in cognitive traits, such as cognitive capacity, memory load, and volume of associated neural tissues (Tello-Ramos et al., 2019). Overall, the processes that underlie variation in cognitive

traits are still poorly understood and it is unclear how cognitive traits may interact with each other to affect fitness-related behaviors.

Another group of cognitive processes that might be highly beneficial are called executive control functions (also known as “executive functions” or “cognitive control”). These are categorized as “higher-order” processes because they act on multiple other cognitive processes, often integrating different types of traits to achieve specific, goal-oriented behaviors (Diamond, 2013; Katz & Wright, 2021; Shettleworth, 2010). In humans, executive control functions enable a diverse range of complex behaviors related to inhibition (i.e., suppression of task-irrelevant information, memories, or behavioral responses), cognitive flexibility (i.e., switching contingencies in response to environmental changes), working memory (i.e., simultaneously holding information in mind and manipulating it), and relational or abstract reasoning (i.e., the ability to learn rules based on the relationships between stimuli and apply them to other relevant contexts) (Diamond, 2013; Lazareva & Wasserman, 2017). Although executive control functions have historically been considered unique to humans (Katz et al., 2007; Premack, 1978), mounting recent evidence suggests that nonhuman animals are capable of complex cognitive processes, including rule-learning (Murphy et al., 2008), cognitive flexibility (Cauchoix et al., 2017; Gonzalez et al., 1967; Tello-Ramos et al., 2018, 2019), and abstract (i.e., relational)-concept learning (Aellen et al., 2022; Hoeschele et al., 2012; Katz & Wright, 2006; Premack, 1983). Yet there are few field studies that explore higher-order cognitive traits in wild populations, and little is known about the ecological and evolutionary processes that may give rise to them.

My dissertation aims to better understand to what extent cognitive traits interact to give rise to information-updating and foraging-based behaviors in a food-caching passerine living across an elevational gradient in the Sierra Nevada. I specifically explore to what extent variation in spatial learning and memory is associated with variation in environmental sampling strategies, cognitive flexibility, and rule-learning in wild, food-caching mountain chickadees (*Poecile gambeli*). Food-caching species, such as many Parids and Corvids, present an excellent and well-studied system to explore cognitive variation. Food-caching is a common behavioral strategy in which individuals living in seasonal environments store extra food when resources are plentiful to retrieve later when resources are scarce, such as during winter (Vander Wall, 1990). Food-caching species typically have highly specialized spatial learning and memory abilities associated with cache retrieval (Pravosudov & Roth, 2013; Vander Wall, 1990), and better cache recovery associated with better spatial cognition is expected to lead to better survival (Pravosudov & Roth, 2013; Sherry et al., 1989). In addition, in most food-caching species, spatial cognition is expected to only be involved with overwinter survival, rather than mate-seeking efforts (Branch et al., 2019). Spatial cognition in Parids has been well studied, and previous evidence in a long-term study of mountain chickadees suggests that variation in spatial learning and memory abilities associated with cache retrieval is associated with differences in environmental harshness (Croston et al., 2016, 2017), is heritable (Croston et al., 2015), has a genetic basis (Branch et al., 2022), is under selection in harsh environments (Sonnenberg et al., 2019), and may be constrained by a proactive interference-mediated tradeoff with cognitive flexibility (Tello-Ramos et al., 2019).

Although learning and memory are two distinct cognitive traits, they will frequently be described together as “learning and memory ability/abilities” throughout this dissertation. Learning refers to the process of forming neural representations of new information, or, according to Shettleworth (2010) as “a change in state due to experience.” Memory, in contrast, describes the retrieval (i.e., recall) of stored representations from long or short-term memory (Shettleworth, 2010). However, these two traits are highly related, and it is often difficult or infeasible to quantify to what extent an individual has learned an association without invoking memory recall. As such, this dissertation will frequently use the term “learning and memory abilities” to suggest that the behavior or performance in question is related to both traits.

Chapter Summaries

This dissertation includes five chapters focused on avian cognition and behavior in wild, food-caching mountain chickadees (*Poecile gambeli*):

Chapter 1: Elevation-related differences in annual survival of adult food-caching mountain chickadees are consistent with natural selection on spatial cognition

Are survival rates of adult mountain chickadees across an elevational gradient consistent with the predicted effects of natural selection on winter-survival related traits? I used a Bayesian hierarchical model to compare survival and detectability rates across an

elevational gradient that correlates with winter environmental harshness. Published as (Benedict et al., 2020).

Chapter 2: Variation in sampling behavior is associated with general rather than local environmental conditions, age, and cognitive abilities

What factors drive individual variation in sampling behaviors associated with information-maintenance? To what extent does the value of sampled information vary with environmental conditions? I applied a measure of evenness to estimate the breadth of resource-use within an experimental feeder array and analyzed variation in resource-use using a linear mixed effects modelling approach. Published as (Benedict et al., 2021).

Chapter 3: Food-caching chickadees do not exhibit directional bias when learning a spatial task

Do chickadees show passive directional biases while searching for food during spatial cognitive tasks? Does individual variation in the direction or degree of directional bias suggest that lateralized cognitive processes drive variation in search strategies? I analyzed foraging decisions made by chickadees during two spatial cognitive tasks to explore whether individuals were more likely to search for food in a given direction (i.e., clockwise versus counterclockwise) around a square feeder array. I used a generalized mixed effects modeling approach to explore to what extent the strength or direction of

individual bias was correlated with individual performance on each task. Published as (Benedict, Heinen, Sonnenberg, Pitera, et al., 2023).

Chapter 4: Learning predictably changing spatial patterns across days in a food-caching bird

How does cognitive flexibility relate to an individual's ability to learn the rule associated with an alternating pattern of rewarding food sources? Can food-caching chickadees learn and use a rule to predict which location will be rewarding next? I conducted two modified serial reversal learning tasks in which a food reward alternated every day between one of two locations within an eight-position, square feeder array. I used generalized linear mixed effects models to (a) compare performance across days, (b), explore to what extent birds could learn the serial reversal rule, (c) assess whether birds learned to use the rule to predict the correct location of the food reward after each switch, and (d) assess to what extent performance correlated with individual cognitive performance on a spatial learning and memory task and a single reversal learning task. Published as (Benedict, Heinen, Sonnenberg, Bridge, et al., 2023).

Chapter 5: Food-caching mountain chickadees can learn abstract rules to solve a complex spatial-temporal pattern

Can food-caching mountain chickadees learn and use an abstract rule to solve a complex spatial-temporal task within a foraging context? Can chickadees learn to use this

rule to predict the next location across days? I designed and conducted a novel spatial-temporal task in which the location of a food reward moved every day in a consistent direction around a square feeder array. I used generalized linear mixed effects models to assess (a) whether individual chickadees could learn the abstract (i.e., relational) direction rule, (b) whether chickadees could learn to use this rule to predict the next location of the food reward and achieve perfect performance, and (c) to what extent performance on the spatial-temporal task was associated with individual spatial learning and memory ability or single reversal learning performance. Published as (Benedict, Heinen, Welklin, et al., 2023).

References

- Aellen, M., Siebeck, U. E., & Bshary, R. (2022). Cleaner wrasse *Labroides dimidiatus* perform above chance in a “matching-to-sample” experiment. *PLOS ONE*, *17*(1), e0262351. <https://doi.org/10.1371/journal.pone.0262351>
- Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Bridge, E. S., & Pravosudov, V. V. (2023). Learning predictably changing spatial patterns across days in a food-caching bird. *Animal Behaviour*, *196*, 55–81. <https://doi.org/10.1016/j.anbehav.2022.11.005>
- Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., & Pravosudov, V. V. (2023). Food-caching chickadees do not exhibit directional bias when learning a spatial task. *Behavioral Ecology and Sociobiology*, *77*(1), 4. <https://doi.org/10.1007/s00265-022-03275-6>
- Benedict, L. M., Heinen, V. K., Welklin, J. F., Sonnenberg, B. R., Whitenack, L. E., Bridge, E. S., & Pravosudov, V. V. (2023). Food-caching mountain chickadees can learn abstract rules to solve a complex spatial-temporal pattern. *Current Biology*, *33*(15), 3136–3144.e5. <https://doi.org/10.1016/j.cub.2023.06.036>
- Benedict, L. M., Pitera, A. M., Branch, C. L., Kozlovsky, D. Y., Sonnenberg, B. R., Bridge, E. S., & Pravosudov, V. V. (2020). Elevation-related differences in annual survival of adult food-caching mountain chickadees are consistent with natural selection on spatial cognition. *Behavioral Ecology and Sociobiology*, *74*, 40. <https://doi.org/10.1007/s00265-020-2817-2>
- Benedict, L. M., Pitera, A. M., Branch, C. L., Sonnenberg, B. R., Heinen, V. K., Bridge, E. S., & Pravosudov, V. V. (2021). Information maintenance of food sources is associated with environment, spatial cognition and age in a food-caching bird. *Animal Behaviour*, *182*, 153–172. <https://doi.org/10.1016/j.anbehav.2021.10.009>
- Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Sonnenberg, B. R., Benedict, L. M., & Pravosudov, V. V. (2019). Elevation-related differences in the age structure of breeding birds suggest stronger selection at harsher elevations. *Behavioral Ecology and Sociobiology*, *73*, 143. <https://doi.org/10.1007/s00265-019-2750-4>
- Branch, C. L., Semenov, G. A., Wagner, D. N., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., Taylor, S. A., & Pravosudov, V. V. (2022). The genetic basis of spatial cognitive variation in a food-caching bird. *Current Biology*, *32*(1), 210–219.e4. <https://doi.org/10.1016/j.cub.2021.10.036>
- Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews*, *78*(4), 575–595. <https://doi.org/10.1017/S1464793103006158>

- Cauchoix, M., Hermer, E., Chaine, A. S., & Morand-Ferron, J. (2017). Cognition in the field: Comparison of reversal learning performance in captive and wild passerines. *Scientific Reports*, 7(1), 12945. <https://doi.org/10.1038/s41598-017-13179-5>
- Croston, R., Branch, C. L., Kozlovsky, D. Y., Dukas, R., & Pravosudov, V. V. (2015). Heritability and the evolution of cognitive traits. *Behavioral Ecology*, 26(6), 1447–1459. <https://doi.org/10.1093/beheco/arv088>
- Croston, R., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Bridge, E. S., Parchman, T., & Pravosudov, V. V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123, 139–149. <https://doi.org/10.1016/j.anbehav.2016.10.004>
- Croston, R., Kozlovsky, D. Y., Branch, C. L., Parchman, T. L., Bridge, E. S., & Pravosudov, V. V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behaviour*, 111, 225–234. <https://doi.org/10.1016/j.anbehav.2015.10.015>
- Dall, S. R. X., Giraldeau, L.-A., Olsson, O., McNamara, J. M., & Stephens, D. W. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, 20(4), 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>
- Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, 64(1), 135–168. <https://doi.org/10.1146/annurev-psych-113011-143750>
- Dukas, R. (2004). Evolutionary Biology of Animal Cognition. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 347–374. <https://doi.org/10.1146/annurev.ecolsys.35.112202.130152>
- Garcia, M. J., Cronin, A., Bowling, T., Bushera, H., Hunter, K. L., & Taylor, R. C. (2019). Dueling frogs: Do male green tree frogs (*Hyla cinerea*) eavesdrop on and assess nearby calling competitors? *Behavioral Ecology and Sociobiology*, 73(2), 21. <https://doi.org/10.1007/s00265-018-2632-1>
- Gonzalez, R. C., Behrend, E. R., & Bitterman, M. E. (1967). Reversal Learning and Forgetting in Bird and Fish. *Science*, 158(3800), 519–521. <https://www.jstor.org/stable/1722549>
- Hoeschele, M., Cook, R. G., Guillette, L. M., Hahn, A. H., & Sturdy, C. B. (2012). Auditory same/different concept learning and generalization in Black-Capped Chickadees (*Poecile atricapillus*). *PLoS ONE*, 7(10), e47691. <https://doi.org/10.1371/journal.pone.0047691>
- Hutchins, E. (2010). Cognitive Ecology. *Topics in Cognitive Science*, 2(4), 705–715. <https://doi.org/10.1111/j.1756-8765.2010.01089.x>

- Katz, J. S., & Wright, A. A. (2006). Same/different abstract-concept learning by pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, *32*(1), 80–86. <https://doi.org/10.1037/0097-7403.32.1.80>
- Katz, J. S., & Wright, A. A. (2021). Issues in the comparative cognition of same/different abstract-concept learning. *Current Opinion in Behavioral Sciences*, *37*, 29–34. <https://doi.org/10.1016/j.cobeha.2020.06.009>
- Katz, J. S., Wright, A. A., & Bodily, K. D. (2007). Issues in the comparative cognition of abstract-concept learning. *Comparative Cognition & Behavior Reviews*, *2*, 79–92. <https://doi.org/10.3819/ccbr.2008.20005>
- Lazareva, O. F., & Wasserman, E. A. (2017). Categories and Concepts in Animals. In *Learning and Memory: A Comprehensive Reference* (pp. 111–139). Elsevier. <https://doi.org/10.1016/B978-0-12-809324-5.21008-0>
- Morand-Ferron, J. (2017). Why learn? The adaptive value of associative learning in wild populations. *Current Opinion in Behavioral Sciences*, *16*, 73–79. <https://doi.org/10.1016/j.cobeha.2017.03.008>
- Morand-Ferron, J., Cole, E. F., & Quinn, J. L. (2016). Studying the evolutionary ecology of cognition in the wild: A review of practical and conceptual challenges. *Biological Reviews*, *91*, 367–389. <https://doi.org/10.1111/brv.12174>
- Morand-Ferron, J., & Quinn, J. L. (2015). The evolution of cognition in natural populations. *Trends in Cognitive Sciences*, *19*, 235–237. <https://doi.org/doi.org/10.1016/j.tics.2015.03.005>
- Murphy, R. A., Mondragon, E., & Murphy, V. A. (2008). Rule learning by rats. *Science*, *319*, 1849–1851. <https://doi.org/10.1126/science.1151564>
- Pravosudov, V. V. (2022). Cognitive ecology in the wild—Advances and challenges in avian cognition research. *Current Opinion in Behavioral Sciences*, *45*, 101138. <https://doi.org/10.1016/j.cobeha.2022.101138>
- Pravosudov, V. V., & Roth, T. C., II. (2013). Cognitive ecology of food hoarding: The evolution of spatial memory and the hippocampus. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 173–193. <https://doi.org/10.1146/annurev-eolsys-110512-135904>
- Premack, D. (1978). On the abstractness of human concepts: Why it would be difficult to talk to a pigeon. In S. H. Hulse, H. Fowler, & W. K. Honig (Eds.), *Cognitive Processes in Animal Behavior* (1st ed., pp. 423–451). Erlbaum.
- Premack, D. (1983). Animal Cognition. *Annual Review of Psychology*, *34*(1), 351–362. <https://doi.org/10.1146/annurev.ps.34.020183.002031>

- Sherry, D. F., Vaccarino, A. L., Buckenham, K., & Herz, R. S. (1989). The hippocampal complex of food-storing birds. *Brain, Behavior and Evolution*, *34*, 308–317.
- Shettleworth, S. J. (2010). *Cognition, Evolution, and Behavior* (2nd ed.). Oxford University Press.
- Sonnenberg, B. R., Branch, C. L., Pitera, A. M., Bridge, E. S., & Pravosudov, V. V. (2019). Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, *29*(4), 670–676.
<https://doi.org/10.1016/j.cub.2019.01.006>
- Stephens, D. W. (2008). Decision ecology: Foraging and the ecology of animal decision making. *Cognitive, Affective, & Behavioral Neuroscience*, *8*(4), 475–484.
<https://doi.org/10.3758/CABN.8.4.475>
- Tello-Ramos, M. C., Branch, C. L., Kozlovsky, D. Y., Pitera, A. M., & Pravosudov, V. V. (2019). Spatial memory and cognitive flexibility trade-offs: To be or not to be flexible, that is the question. *Animal Behaviour*, 129–136.
<https://doi.org/10.1016/j.anbehav.2018.02.019>
- Tello-Ramos, M. C., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., & Pravosudov, V. V. (2018). Memory in wild mountain chickadees from different elevations: Comparing first-year birds with older survivors. *Animal Behaviour*.
<https://doi.org/10.1016/j.anbehav.2017.12.019>
- Vander Wall, S. B. (1990). *Food hoarding in animals*. University of Chicago Press.

Chapter 1 Elevation-Related Differences in Annual Survival of Adult Food-Caching Mountain Chickadees Are Consistent with Natural Selection on Spatial Cognition

L.M. Benedict^{1*}, A.M. Pitera¹, C.L. Branch², D.Y. Kozlovsky³, B.R. Sonnenberg¹, E.S. Bridge⁴, V.V. Pravosudov¹.

¹ Department of Biology and Ecology, Evolution and Conservation Biology Graduate Program, University of Nevada, Reno, Reno, NV, U.S.A.; ² Cornell Lab of Ornithology, Cornell University, Ithaca, NY, U.S.A.; ³ Department of Biology, Villanova University, Villanova, PA, U.S.A.; ⁴ University of Oklahoma, Oklahoma Biological Survey, Norman, OK, U.S.A.

*Corresponding author, Lauren Benedict Nguyen, published as Lauren M. Benedict

Publication Citation

Benedict, L. M., Pitera, A. M., Branch, C. L., Kozlovsky, D. Y., Sonnenberg, B. R., Bridge, E. S., & Pravosudov, V. V. (2020). Elevation-related differences in annual survival of adult food-caching mountain chickadees are consistent with natural selection on spatial cognition. *Behavioral Ecology and Sociobiology*, 74:40. [doi: 10.1007/s00265-020-2817-2](https://doi.org/10.1007/s00265-020-2817-2)

Abstract

Animals inhabiting montane gradients experience varying winter climates that may result in differential selection on survival-related traits. Higher elevations in temperate climates are characterized by harsher winters with greater and longer-lasting snow cover compared to lower elevations, potentially leading to stronger selection for traits that improve fitness under these harsher conditions. For food-caching mountain chickadees, *Poecile gambeli*, inhabiting harsh high elevation environments, individual variation in spatial cognitive abilities related to cache retrieval is associated with significant differences in overwinter survival. Compared to lower elevations, stronger predicted selection on traits needed for overwinter survival at higher elevations can be expected to result in higher adult annual survival despite harsher environmental conditions, indicating that individuals that survive their first winter are better suited to survive similar subsequent selection events. Here, we used a Bayesian hierarchical Cormack-Jolly-Seber (CJS) model to estimate and compare survival of adult mountain chickadees at higher and lower elevations over three years. We showed that adult survival was consistently higher at higher elevations despite much harsher environmental conditions, supporting our hypothesis that selection on overwinter survival related traits (such as spatial cognition) is stronger at high elevation study area than at lower elevation.

Significance Statement

Understanding how environmental conditions are associated with different selection strengths on survival-related traits is an important question in behavioral ecology. Directly estimating differences in strength of selection is daunting, but comparing survival between environments may provide an alternative method. We tested for differences in adult survival in a resident food-caching species at higher and lower elevations varying in winter climate severity. These birds rely on food caches for winter survival and juvenile birds with better spatial cognition (needed for cache retrieval) have higher survival during their first year at higher harsher elevations. Here, we report higher adult survival at higher elevations compared to lower elevations, despite much harsher winter environment. Such findings support our hypothesis for stronger selection in harsher winter conditions because individuals that survive their first year under stronger selection are better suited to survive subsequent selection events.

Introduction

The neural processes involved with the acquisition, retention, storage, and use of information, generally referred to as cognition (Dukas 2004), give rise to a wide range of observable behaviors such as foraging decisions, predator avoidance and mate selection (Morand-Ferron et al., 2016). Variation in cognition within populations has been attributed to short-term evolutionary processes (i.e., selection; Morand-Ferron & Quinn 2015), but evidence in natural populations has been limited due to various difficulties inherent in measuring cognitive traits in large groups of animals in the wild (Morand-Ferron et al., 2016).

The evolution of phenotypes by natural selection may occur via differential reproductive success or differential mortality (Endler 1986). In designing a field study to explore how selection may give rise to variation in cognition, the choice of species, study population and cognitive test must all be carefully considered to ensure that the expected relationships are relevant and measurable given the selective agent (i.e., the environment) and the cognitive trait in question (Morand-Ferron et al., 2016). Food-caching species thus present a good system because individuals depend on previously cached stores of food as a primary food source during winter (Vander Wall 1990). Many species rely on cognition to locate previously made caches; more caching and better cache recovery via spatial cognition is expected to directly lead to better survival (Sherry et al., 1989; Pravosudov & Roth 2013). In addition, in most food-caching species, spatial cognition is expected to only be involved with overwinter survival, rather than reproduction (Branch et al., 2019a).

In temperate climates, winter is typically the period of highest mortality for resident small-bodied endotherms and selection is expected to act on traits related to winter survival (Lack 1954; Jansson et al., 1981). Winter conditions such as snow depth and duration of snow cover typically increase in severity with increasing elevation, creating a montane gradient of environmental harshness with rapidly deteriorating conditions above a certain threshold (e.g., snowline) elevation (Royce and Barbour, 2001). This in turn likely leads to the differential survival of individuals carrying certain phenotypes and provides the opportunity to investigate different strengths of selection on survival-related traits within a small geographic area. Furthermore, first-year animals across many taxa are less likely to survive winter than adults, with some species experiencing up to 70% post-dispersal juvenile overwinter mortality (Munch 2003). When there is strong selection on winter survival-related traits, juvenile individuals that survive their first winter and reach their first reproductive season (e.g., becoming adults) are expected to have higher probabilities of survival during subsequent winters (Reznick et al., 1996). This may lead to a difference in survival probability by age-class – higher mortality of juveniles, but higher survival of adults (Reznick et al., 1996).

In our long-term study of food-caching mountain chickadees (*Poecile gambeli*), we previously demonstrated directional natural selection on spatial cognition at higher elevations in the Sierra Nevada characterized by harsher winter conditions and longer lasting snow cover (Sonnenberg et al., 2019). At high elevations, juvenile chickadees that performed better on a spatial learning and memory task were more likely to survive their first winter, becoming adults, while those that performed worse on the spatial task died before maturity (Sonnenberg et al., 2019a). Furthermore, we previously found significant

cognitive, morphological and behavioral differences between chickadees inhabiting different elevations that differ substantially in environmental harshness: chickadees at harsher, higher elevations were shown to have better spatial learning and memory abilities (in the lab: Freas et al., 2012; and in the wild: Croston et al., 2016, 2017; Tello-Ramos et al., 2018), larger hippocampi with higher total number of neurons (Freas et al., 2013), higher rates of caching (in the lab: Freas et al., 2012) and a greater mortality of first-year juveniles during their first winter (Branch et al., 2019b) compared to chickadees from milder, lower elevations. Winter conditions are always harsher at higher elevations than at lower elevations in any given year at our study area (Kozlovsky et al., 2018). If birds from both elevations were phenotypically the same, birds at harsher higher elevations would be expected to have lower survival due to harsher conditions. However, previous research showed that birds at high elevations cache more food and have better spatial cognition compared to birds at lower elevation (Freas et al., 2012, 2013) and we previously argued that such differences are a product of natural selection resulting in higher elevation birds being adapted to survival in harsher conditions. Based on these elevation-related differences, we hypothesize that natural selection on spatial cognition may be stronger at higher elevations than at lower ones, likely due to greater dependence on successful cache recovery needed to survive harsher winters.

In addition to previously demonstrating selection on cognition at higher elevations (Sonnenberg et al., 2019), we have also found indirect evidence for this hypothesis in that juvenile survival during their first winter is lower at high elevation (Branch et al., 2019b). Given these findings, if selection is stronger at high elevation, resulting in an adult population that has relatively better spatial cognition and so is well-adapted to survive

harsh winter conditions, we would also expect these adults to show higher survival during subsequent years compared to adults from lower elevations with relaxed selection on cognitive traits. If selection is relaxed at lower elevations, adult survival would be more similar to that of juveniles at the same elevation, as it may be affected by a variety of factors including occasional and unpredictable winter events, predation, etc.

Here we present new data on annual survival rates of adult mountain chickadees to test our prediction that given stronger selection during the first winter of life, adults should have higher annual survival at higher elevations despite much harsher winter conditions. We use our long-term study area situated across an elevational gradient snowline in the Sierra Nevada, such that the difference in environmental harshness between our high and low elevation areas is substantial and ecologically relevant (Croston et al., 2017b; Royce and Barbour, 2001). Our prediction was based on several previous findings: (1) birds at high elevation have better spatial cognition and a larger hippocampus (Freas et al., 2012, 2013) and (2) there is evidence of strong directional natural selection on spatial cognition at higher elevations where only birds with better cognition survive into adulthood (Sonnenberg et al., 2019).

The key detail is that this prediction only concerns survival of adults – once a juvenile survives the strong selection event (e.g., first winter season), the likelihood that the individual (now an adult) will survive subsequent years is higher than expected at low elevation where selection is relaxed. Considering that stronger selection should significantly reduce existing variation in cognitive abilities in juveniles resulting in adults

with superior spatial cognition (e.g., Sonnenberg et al., 2019), these adults can be expected to successfully survive subsequent, albeit harsh, winters.

Methods

Study Site

We trapped and banded mountain chickadees from August 2014 to July 2018 at our long-term study system in Sagehen Experimental Forest, 10 kilometers north of Truckee, CA, U.S.A. in the Sierra Nevada (Branch et al., 2019a; R. Croston et al., 2016; Croston et al., 2017b; Freas et al., 2012; Kozlovsky et al., 2018a; Sonnenberg et al., 2019a; Tello-Ramos et al., 2018). Birds were trapped from two elevations differing in environmental harshness (Kozlovsky et al., 2018b): “low” elevation (ca. 1900 m) and “high” elevation (ca. 2400 m). Birds were trapped using mistnets set up around established feeders (nonbreeding season) or at one of ca. 350 nestboxes (breeding season) and fitted with a unique color band combination, including a plastic band containing a passive integrated transponder (PIT) tag (IB Technology, Leicestershire, U.K.). Birds were aged when initially processed according to at least one of four metrics: rectrix condition and shape (Meigs et al., 1983), molt of tail or wing feathers (Pyle, 1997), breeding status (Branch et al., 2019a) during capture and our previous records for birds with metal United States Geological Survey issued leg bands (which we previously banded as nestlings).

Sampling Methods

Mountain chickadees are resident passerines with one short post-natal dispersal event and subsequent high site fidelity, and as such could be sampled year-round (Branch et al., 2016). Each 12-month sampling period started after post-natal dispersal (Aug) and ended after the subsequent breeding season (Jul) to ensure that each winter season (ca. Dec-Mar) would not be split between two sampling periods. We constructed a capture-recapture (CR) dataset consisting of one presence observation per individual per sampling period. It was not possible to record data blind because our study involved focal animals in the field. Birds were considered present if (1) captured or re-sighted using a nestbox during our annual breeding surveys (May-July; (Branch et al., 2019b; Kozlovsky et al., 2018b); (2) captured or recaptured during nonbreeding season banding efforts using mistnets at established feeder sites (Aug-Feb); or (3) recorded automatically during the winter at one of four “smart” arrays, each consisting of eight feeders equipped with radio frequency identification (RFID) technology (Bridge & Bonter 2011) and attached to a square frame suspended from trees (R. Croston et al., 2016; Croston et al., 2017b; Pitera et al., 2018). Presence was recorded once per 12-month sampling period regardless of season or number of recaptures, resightings or automated recordings (hereafter all referred to as detections). For example, a bird detected during banding efforts in November 2017 and detected at a smart feeder in January 2018 would be recorded as present once during the 2017-18 sampling period.

We only analyzed birds that were aged as adults at the first sampling period. Detection of juveniles during the non-breeding season at our low elevation site was

insufficient during one winter of the study (2017-18). This gap in detection was likely due to decreased motivation for chickadees to forage from supplemental feeders during mild autumn and winter conditions that might have led to more abundant natural food sources. Only our low elevation site experienced low detection during 2017-18. Adult detection at both sites was buffered by additional opportunity for detection during breeding season. Thus, instead of potentially underestimating juvenile survival at our low elevation site, we only used adult data from both sites for this analysis.

Methods for Estimating Survival

To estimate annual apparent survival (ϕ) and the probability of detection (p), we fit a Bayesian hierarchical, single-state CJS model (Cormack 1964; Jolly 1965; Seber 1965) based on code developed by (Kéry and Schaub, 2011). In this study, apparent survival is the probability that an individual will be alive and present in the sampling period (t) given that it was alive and present during the previous sampling period ($t-1$). We expect that the apparent survival probability is close to the true survival probability in our system because dispersal distances of mountain chickadees are typically short, chickadees are known to be rather sedentary, and we do not appear to have substantial movement in or out of the study area (Branch et al., 2016; Ekman, 1989).

The CJS model makes several assumptions, including that PIT tags are not lost, individuals are not misidentified, the marked and recaptured individuals represent a random sample of the study population, all individuals within groups have the same survival and recapture probabilities, individual survival and detection probabilities are

independent, and captures are instantaneous, meaning that sampling periods are short and birds are released immediately to avoid variation in the intervals between sampling occasions that could introduce variation in survival probabilities. Our study design meets all but the last of these assumptions: due to our year-round sampling scheme, sampling periods were not instantaneous. However, we argue that the biases resulting from this assumption violation are minor compared to those that would result from only using one of the three detection methods we have available. The question driving this analysis is whether there is differential overwinter survival between adults at our two elevation sites that might suggest different strengths of selection as suggested in Branch et al., (2019b). To only use data from breeding season would bias our dataset towards adult birds that have already survived the major selection event (i.e., first winter), possibly resulting in overestimates of survival. To only use data from nonbreeding season banding efforts would bias our dataset towards unbanded adults because we have low rates of recapturing banded adults, possibly underestimating survival. To only use data from automated recordings during winter would limit our sample size to only birds that we have already tagged and that have already survived the initial stages of winter. Thus, we feel it is appropriate to use presence data from all three of these methods to increase the probability of detection (p) and obtain an overall less biased estimate for apparent survival.

Model Likelihood

The model likelihood describes both a state process z , in which the individual chickadee i at sampling period t is either dead ($z_{i,t}=0$) or alive ($z_{i,t}=1$), and an observation process y , in which the individual is either detected ($y_{i,t}=0$) or not detected ($y_{i,t}=1$) during the sampling period. A chickadee's state (i.e., dead or alive) will depend on its state of the previous occasion $z_{i,t-1}$. It naturally follows that a dead chickadee cannot move to any other state than dead. At the same time, an chickadee that is alive in a given year will change states depending on the probability of survival, $\phi_{i,t}$ [Eq. 1.1]. The state of an individual chickadee at first capture z_{i,f_1} will always be 1 because we know the bird is alive when we initially band it [Eq. 1.2]. As such, CJS models are conditioned on first capture, meaning that presence data from the first capture is not treated as survival data in the model because its value is constant for all individuals (Kéry and Schaub, 2011). Hence, we use four years of data (2014-2018) but only report survival estimates for three sampling periods (2015-2018).

$$z_{i,t}|z_{i,t-1} \sim \text{Bernoulli}(z_{i,t-1}\phi_{i,t}) \quad (1.1)$$

$$z_{i,f_1} = 1 \quad (1.2)$$

Whether or not a chickadee is detected $y_{i,t}$ during a sampling period depends on the probability of detection $p_{i,t}$ and the state of the individual $z_{i,t}$, as a bird can only be detected by our methods if it was alive [Eq. 1.3].

$$y_{i,t}|z_{i,t} \sim \text{Bernoulli}(z_{i,t}p_{i,t}) \quad (1.3)$$

Model for Survival

We fit the CR data with a fixed group effect of elevation and a fixed effect of time where β_g is the group effect (β_L or β_H for low and high elevation sites respectively) and γ is the time effect on the apparent survival ϕ of an individual chickadee i at sampling period t [Eq. 1.4].

$$\text{logit}(\phi_{i,t}) = \beta_{g(i)} + \gamma_t \quad (1.4)$$

To avoid an overparameterized model, we determined that γ_t represented the time effect on the first group and set the group effect of lower elevations to zero ($\beta_L = 0$; (Kéry and Schaub, 2011)). We could then evaluate the posterior distribution of β_H as the difference in apparent survival between sites.

Statistical Analysis

All CJS models were fitted in JAGS (version 4.3.0; Plummer, 2003) using R (version 3.5.2; R Development Core Team, 2011) and the R2jags wrapper package

(version 0.5.7; Su and Masanao Yajima, 2015). Goodness-of-fit (GOF) tests were conducted using the R2ucare package (version 1.0.0; Gimenez et al., 2017). The model was first tested for convergence with simulated data. Model fit with observed data was evaluated using the Gelman-Ruben diagnostic, prior posterior overlap (PPO) and by visual inspection of both the posterior distributions of all parameters and data simulated from the joint posterior distribution. Confidence in the difference in probability of survival between elevations was determined by the degree of overlap with zero of the posterior distribution of β_H .

Results

From Aug 2014-Jul 2018, we banded 1161 individual birds (552 at high, 609 at low) of which we detected 415 individuals again (219 at high, 196 at low). Of these, 336 birds were marked as adults when banded (152 at high, 184 at low) and of these we detected 202 individuals again (106 at high, 96 at low). Across all four sampling periods, detections comprised 26.3% of the total observations and 37.5% of all adult observations.

The average annual apparent survival probability varied over time and between sites, with the lowest annual rate in 2016-17 for both sites and consistently higher survival probabilities at the high elevation site (Fig. 1.1; Fig. 1.2). Averaged across years, the survival rate for adults was ca. 10% higher at higher elevations ($\phi = 0.65, 0.54-0.75$) than at lower elevations ($\phi = 0.53, 0.42-0.65$). The 95% credible intervals for the posterior distribution of the difference in annual survival between sites did not overlap

with zero (mean $\beta_H = 0.51$, 0.28-0.99), providing strong support for a nonzero difference in apparent survival between sites (Fig. 1.3). The PPO was 28.1%. Average detection probability was similar between sites but was slightly lower at higher elevations ($p = 0.77$, 0.65-0.87) than at lower elevations ($p = 0.91$, 0.79-0.98; Fig. 1.4).

Discussion

Our findings supported our prediction, that adult chickadees at higher elevations had higher annual survival probabilities across all three years of the study compared to adults from lower elevations. Our previous work showed that the proportion of adult to first-year breeders was greater at higher elevations compared to lower elevations, suggesting greater juvenile mortality during the same three years of sampling at our high elevation site (Branch et al., 2019b). Combined, our current and previous findings suggest that there was a greater difference in survival probabilities by age-class at higher elevations than at lower elevations. These data lend further support for our hypothesis for stronger selection on traits related to winter survival at our harsher, higher elevation site compared to our lower elevation site. When selection on overwinter traits is strong, birds that carry those traits and survive their first winter are more likely to survive subsequent winters with similar conditions. When selection is relaxed, more birds without these traits survive their first winter. This may lead to winter-related mortality of birds lacking these traits later in life as adults, especially in environments with variable winter conditions. Thus, even though winter conditions are much harsher, adults at higher elevations actually have a higher probability of survival compared to adults at milder, lower

elevations because more adults may carry traits that are advantageous to surviving harsh winters. We have previously found evidence for selection on spatial cognition, a trait essential to chickadee survival, at higher elevations (Sonnenberg et al., 2019). From our current findings that the strength of selection varies between higher and lower elevations, we infer that the strength of selection on spatial cognition may also vary between elevations.

The important point in our comparison is not elevation per se but differences in winter climate. This work follows extensive comparison of multiple populations of black-capped chickadees across a winter climate gradient on the continental scale and mountain chickadees at our current field area (Pravosudov & Roth 2013) showing a significant association between winter climate severity and spatial cognition. Following this highly consistent and repeatable association, we focused on mountain chickadees at high and low elevations as a model system to test specific evolutionary questions. Our high and low elevation study areas are situated strategically on opposite sides of the region's "snowline," a fairly narrow threshold elevation above which precipitation typically falls as snow during the intermediate seasons and below which falls as rain (Barbour & Minnich 2000; Kozlovsky et al., 2018; Branch & Pravosudov, 2020). As such, winter conditions do not change continuously and gradually with the elevational gradient, but instead change rather abruptly around this "snowline" elevation; there is a stark difference in environmental harshness between habitats situated on either side of this boundary (Barbour & Minnich 2000). Therefore, we would not expect to see continuous variation in cognition or any other winter survival-related traits. Instead, we would expect to see significant differences across the critical winter climate threshold around the

snowline (Branch & Pravosudov, 2020). Previous research from our lab has found significant differences between birds from each elevation for multiple traits (Freas et al., 2012; 2013; Kozlovsky et al., 2014; Croston et al., 2016; Kozlovsky et al., 2018). This wide suite of ecologically relevant differences between birds from these elevations, across multiple years, suggests that the chosen areas of study are appropriate to answer questions concerned with how differences in environment may give rise to different traits.

The obvious limitation is that our study is limited to a single mountain range because replicating the long-term study system across additional mountain ranges while maintaining the same level of detail would be unrealistic given the resources and effort required to collect data year-round from just one mountain range. Our long-term study system (established in 2011) includes individual data for over a thousand individual mountain chickadees collected from banding efforts, breeding surveys and cognition tests. Although we do not sample discrete plots across different mountain ranges, we instead exhaustively sample a large area (over 200 hectares) at high and low elevations year-round. A philosophical question remains: do studies that do not have clear replication on a larger scale still have value? We argue they do, because such studies may still capture a single event that provides key insights into evolutionary processes. Examples of such studies are widespread in the literature, but one notable example is the pivotal work by Boag & Grant (1981) showing selection on beak size in one species of finch on one island in the Galapagos. Despite lacking replication, this work documented the effects of an event on one population of finches and contributed hugely to the understanding of contemporary evolution.

Annual variation in seasonal harshness and food availability could impact annual survival rates. Among the three years of the study, 2016-17 was characterized by the lowest survival estimates and the most severe winter snow conditions at both high and low elevations, one of the snowiest years in ca. 100 years for the region (Kozlovsky et al., 2018). Survival estimates for the other two years (both characterized by average winter conditions; Kozlovsky et al., 2018 for 2015-16 and unpublished data for 2017-18) were similar, albeit different between elevations. Although our results do not appear to show that the differences among years were significant, this trend suggests that harsh winter conditions may be a primary cause of mortality in our system. Most importantly, survival rates at high elevation were consistently greater than at low elevation for all years, further supporting that even when environmental conditions become more harsh, adult birds at high elevation still have a greater chance of surviving than birds at low elevation. At the same time, winter conditions are always harsher at high elevation in any given year (Kozlovsky et al., 2018). Chickadees are pine seed specialists and depend on food cached during autumn for overwinter survival. However, our rough estimates of various pine seed production in autumn do not appear to be associated with survival rates among the three years. More long-term data would be necessary to detect any potential associations among pine seed production, winter climate and survival.

High overwinter mortality in seasonal environments is likely due to starvation resulting from a paucity of food and can act to regulate populations (Lack, 1954). In our system, daily foraging behaviors differed between elevations, suggesting that risk of starvation may be higher at our high elevation site: birds at higher elevations have foraging behavior that is consistent with higher risk of starvation and greater uncertainty

of food availability while lower elevation birds seem to forage in a manner that is consistent with lower risk of starvation (Pitera et al., 2018). Fluctuating dispersal rates could also assist in regulating population size and were not directly addressed in this study; however, mountain chickadees show high site fidelity after fledging (Ekman, 1989; McCallum et al., 1999) and detection probabilities of adults in the study were high for both sites. Birds in our system have short postnatal dispersal distances at both elevations (ca. 1 km) and despite having banded more than 2,000 nestlings and more than 1,000 birds during the fall and winter, we have not detected a single individual that permanently moved (i.e., detected breeding) between elevations. This suggests that the apparent survival we measured was likely close to the true survival of the population and not the result of dispersal or transience. Interestingly, even though we had more use of RFID feeders at our high elevation site, which could potentially lead to better detection of birds and hence to higher survival estimates, the detection probability was actually slightly higher at our low elevation site. This suggests that our sampling efforts were sufficient.

Predation rates were also not directly assessed in this study and could contribute to the difference in adult survival between sites. However, if the greater mortality we found at lower elevations was caused by higher predation rates, we would expect to also find greater mortality of juveniles, as juveniles are often less experienced and more vulnerable to predation (Kershner et al., 2004). We would also expect to detect differences between elevations in nest abandonment during breeding season, which could be indicative of predation (Wood, 2002). However, the number of abandoned nests with eggs or nestlings did not differ between elevations (Branch et al., 2019a) arguing against

potential significant differences between elevations in predation, at least during the breeding period. As this was not the case, we believe that predation likely played a negligible role in the detected differences between survival rates.

In summary, our results demonstrate that adult food-caching chickadees have higher annual survival at higher elevations compared to lower elevations despite more severe winter conditions. Coupled with our previous findings of significantly lower juvenile survival at higher elevation compared to low elevation across the same three years (Branch et al 2019b), our results support the hypothesis for stronger selection on survival-related traits (i.e., spatial cognition) at higher elevations. Although we did not directly compare selection on spatial cognition between elevations and only demonstrated strong directional selection at higher elevations, such contrasts in annual survival rates between age classes (juveniles versus adults) are what we would expect given differences in the strengths of selection pressures. Spatial cognition is essential for survival in mountain chickadees inhabiting harsh conditions (Sonnenberg et al., 2019) and our recent findings of lower juvenile survival but higher adult survival at harsh high elevations further support our previous findings that spatial cognition is under natural selection (Sonnenberg et al., 2019).

Acknowledgements

We thank Rebecca Croston and Maria Tello-Ramos, who also participated in data collection. Thank you to Jeff Brown and staff of Sagehen Experimental Forest field station (University of California Berkeley) for assistance at our field site. Thank you to Perry Williams (University of Nevada, Reno) and Noah Benedict for advice in designing and implementing the Bayesian model. We thank Associate Editor (Dr. Dustin Rubenstein) and two anonymous reviewers for constructive criticisms and suggestions resulting in a significantly improved manuscript.

Ethical statement

To the best of our knowledge, no birds were harmed by the collection of this data. All procedures were in accordance with the University of Nevada, Reno Institutional Animal Care and Use Committee (protocol no. 00046 and 00603) and California Department of Fish and Wildlife Permit SC-5210 (DocID: D-0019571790-9).

Data Accessibility

The data supporting this study are publicly available via GitHub at <https://github.com/LMbenedit/Publications/>.

Author Contributions

LMB, AMP, CLB, DYK, BRS, and VVP collected data during breeding and nonbreeding seasons across four years. ESB designed the RFID system and provided support throughout data collection. AMP coalesced data from multiple sources to construct presence dataset later used by LMB to construct the CR dataset. LMB conducted all analyses. LMB and VVP wrote the first draft of the manuscript. AMP, CLB, DYK and BRS all contributed to the writing. VVP established the field system.

Competing Interests

The authors report no competing interests.

Funding

This research was supported by the National Science Foundation (NSF) grant IOS1351295 and IOS1856181 to VVP and Division of Biological Infrastructure grant 1556313 to ESB. CLB was supported by the NSF Doctoral Dissertation Improvement Grant 1600845.

References

- Barbour MG, Minnich RA (2000). Californian upland forests and woodlands, Pg 16-202 in: M.G. Barbour and W.D. Billings (eds.), North American terrestrial vegetation, 2nd edition, Cambridge University Press, New York.
- Boag PT, Grant PR (1981). Intense natural selection in a population of Darwin's finches (*Geospizinae*) in the Galapagos. *Science* 214:82-5.
<https://doi.org/10.1126/science.214.4516.82>
- Branch CL, Pravosudov VV (2020). Variation in song structure along an elevation gradient in a resident songbird. *Behavioral Ecology and Sociobiology*, 74:9.
<https://doi.org/10.1007/s00265-019-2786-5>
- Branch CL, Kozlovsky DY, Croston R, Pitera AM, Pravosudov VV (2016). Mountain chickadees return to their post-natal dispersal settlements following long-term captivity. *Behavior*, 153:551–67. <https://doi.org/10.1163/1568539x-00003363>
- Branch CL, Pitera AM, Kozlovsky DY, Bridge E, Pravosudov VV (2019a). Smart is the new sexy: Female mountain chickadees increase reproductive investment when mated to males with better spatial cognition. *Ecology Letters*, 22:897-903.
<https://doi.org/10.1111/ele.13249>
- Branch CL, Pitera AM, Kozlovsky DY, Sonnenberg BR, Benedict LM, Pravosudov VV (2019b). Elevation-related differences in the age structure of breeding birds suggest stronger selection at harsher elevations. *Behavioral Ecology and Sociobiology*, 73:143. <https://doi.org/10.1007/s00265-019-2750-4>
- Bridge ES, Bontar DN (2011). A low-cost radio frequency identification device for ornithological research. *Journal of Field Ornithology*, 82:52–59
- Cormack RM (1964) Estimates of survival from the sighting of marked animals. *Biometrika*, 51:429-438. <https://doi.org/10.1093/biomet/51.3-4.429>
- Croston R, Kozlovsky DY, Branch CL, Parchman TL, Bridge ES, Pravosudov VV (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behaviour*, 111:225–3
<https://doi.org/10.1016/j.anbehav.2015.10.015>
- Croston R, Branch CL, Pitera AM, Kozlovsky DY, Bridge ES, Parchman TL, Pravosudov VV (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123:139–49 <https://doi.org/10.1016/j.anbehav.2016.10.004>.
- Ekman J (1989). Ecology of non-breeding social systems of Parus. *The Wilson Bulletin* (Wilson Ornithol Society) 101: 263–88.

- Endler JA (1986). *Natural Selection in the Wild*. Monographs in Population Biology 21. Ed. R. M. May. Princeton (NJ): Princeton Univ Press.
- Freas CA, LaDage LD, Roth TC, Pravosudov VV (2012). Elevation-related differences in memory and the hippocampus in mountain chickadees, *Poecile gambeli*. *Animal Behaviour*, 84:121–27 <https://doi.org/10.1016/j.anbehav.2012.04.018>
- Freas CA, Bingman K, LaDage LD, Pravosudov VV (2013). Untangling elevation-related differences in the hippocampus in food-caching mountain chickadees: The effect of a uniform captive environment. *Brain, Behavior Evolution*, 3:199-209 <https://doi.org/10.1159/000355503>
- Gimenez O, Lebreton J, Choquet R, Pradel R (2017). R2ucare: goodness-of-fit tests for capture-recapture models <https://cran.r-project.org/package=R2ucare>
- Jansson C, Ekman J, von Brömssen A (1981). Winter mortality and food supply in Tits *Parus spp.* *Nordic Society Oikos*, 37:313-322.
- Jolly GS (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika*, 52:225-248 <https://doi.org/10.1093/biomet/52.1-2.225>
- Kershner EL, Walk JW, Warner RE (2004). Postfledging movements and survival of juvenile eastern meadowlarks (*Sturnella magna*) in Illinois. *Auk* 121:1146–54
- Kéry M, Schaub M (2011). Estimation of survival from capture–recapture data using the Cormack–Jolly–Seber model. *Bayesian Popul Anal Using WinBUGS*. <https://doi.org/10.1016/b978-0-12-387020-9.00007-9>
- Kozlovsky DY, Branch CL, Freas CA, Pravosudov VV (2014). Elevation-related differences in novel exploration and social dominance in food-caching mountain chickadees. *Behavioral Ecology and Sociobiology*, 68:1871-1881.
- Kozlovsky DY, Branch CL, Pitera AM, Pravosudov VV (2018) Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *Royal Society Open Science*, 5 <https://doi.org/10.1098/rsos.171604>
- Lack D (1954). *The Natural Regulation of Animal Numbers*. Oxford University Press.
- McCallum DA, Grundel R, Dahlsten DL (1999). Mountain chickadee (*Poecile gambeli*), *The Birds of North America Online*, A. Poole Ed. Ithaca: Cornell Lab of Ornithol
- Meigs JB, Smith DC, Van Buskirk J (1983). Age determination of black-capped chickadees. *Journal of Field Ornithology*, 54:283–86
- Morand-Ferron J, Quinn, JL (2015). The evolution of cognition in natural populations. *Trends in Cognitive Sciences*, 19:235–237.

- Morand-Ferron J, Cole EF, Quinn JL (2016). Studying the evolutionary ecology of cognition in the wild: a review of practical and conceptual challenges. *Biological Reviews*, 91:367–389.
- Munch SB, Mangel M, Conover DO (2003). Quantifying natural selection on body size from field data: Winter mortality in *Menidia menidia*. *Ecology*, 84:2168–77 <https://doi.org/10.1890/02-0137>
- Pitera AM, Branch CL, Bridge ES, Pravosudov VV (2018). Daily foraging routines in food-caching mountain chickadees are associated with variation in environmental harshness. *Animal Behaviour*, 143:93-104 [10.1016/j.anbehav.2018.07.011](https://doi.org/10.1016/j.anbehav.2018.07.011)
- Plummer M (2003). JAGS: A program for analysis of Bayesian graphical models using Gibbs Sampling. DSC 2003 Working Papers, <http://www.ci.tuwien.ac.at/Conferences/DSC-2003/>
- Pravosudov VV, Roth TC (2013). Cognitive ecology of food hoarding: The Evolution of spatial memory and the hippocampus. *Annual Review of Ecology, Evolution and Systematics*, 44:173–93 <https://doi.org/10.1146/annurev-ecolsys-110512-135904>
- Pyle P (1997). Molt Limits in North American Passerines. *North American Bird Bander*, 22:49–89.
- R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, <https://doi.org/10.1007/978-3-540-74686-7>
- Reznick DN, Butler MJ, Rodd FH, Ross P (1996). Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution*, 50:1651 <https://doi.org/10.2307/2410901>
- Royce EB, Barbour MG (2001). Mediterranean climate effects. I. Conifer water use across a Sierra Nevada ecotone. *American Journal of Botany*, 88:911–18 <https://doi.org/10.2307/2657044>
- Seber GAF (1965). A note on the multiple-recapture census. *Biometrika*, 52:249-260 <https://doi.org/10.1093/biomet/52.1-2.249>
- Sherry DF, Vaccarino AL, Buckenham K, Herz RS (1989). The hippocampal complex of food-storing birds. *Brain, Behavior Evolution*, 34:308–17 <https://doi.org/10.1159/000116516>
- Sonnenberg BR, Branch CL, Pitera AM, Bridge ES, Pravosudov V (2019). Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, 29:670-676.e3 <https://doi.org/10.1016/j.cub.2019.01.006>

Su Y, Yajima M (2015). R2jags: Using R to run “JAGS”
<https://cran.project.org/package=R2jags>

Tello-Ramos MC, Branch CL, Pitera AM, Kozlovsky DY, Bridge ES, Pravosudov VV
(2018). Memory in wild mountain chickadees from different elevations:
Comparing first-year birds with older survivors. *Animal Behaviour*, 137:149–60
<https://doi.org/10.1016/j.anbehav.2017.12.019>

Vander Wall SB (1990). *Food hoarding in animals*. Chicago: University of Chicago Press

Williams GE, Wood PB (2002). Are traditional methods of determining nest predators
and nest fates reliable? An experiment with wood thrushes (*Hylocichla mustelina*)
using miniature video cameras. *Auk*, 119:1126–32
<https://doi.org/10.2307/4090242>

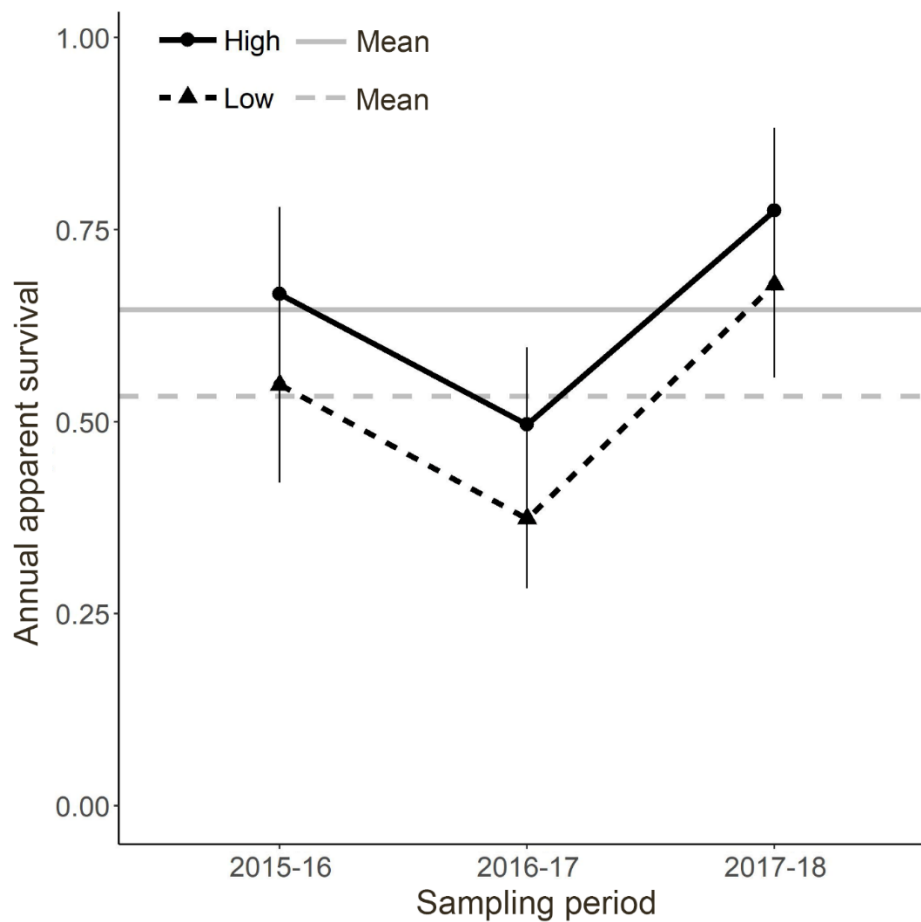
Figures*Figure 1.1*

Figure 1.1. Annual apparent survival probability of adult chickadees at our high and low elevation sites by year 2015-2018. Solid lines and circles indicate apparent survival at the high elevation site. Dashed lines and triangles indicate apparent survival at the low elevation site. Grey lines indicate the mean for each elevation.

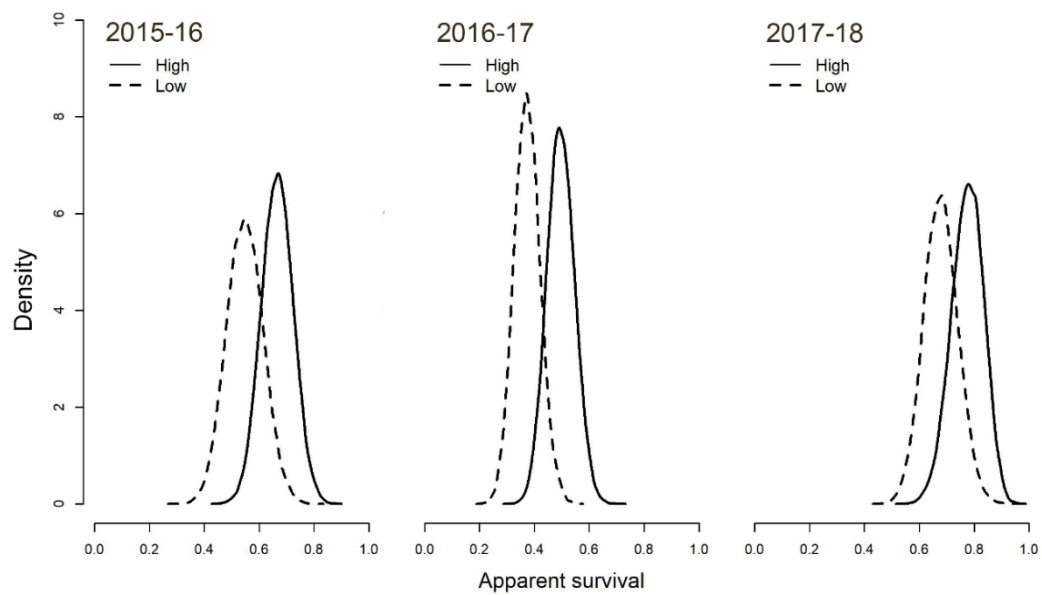
Figure 1.2**Figure 1.2.** Posterior distributions of apparent survival per sampling period and site.

Figure 1.3

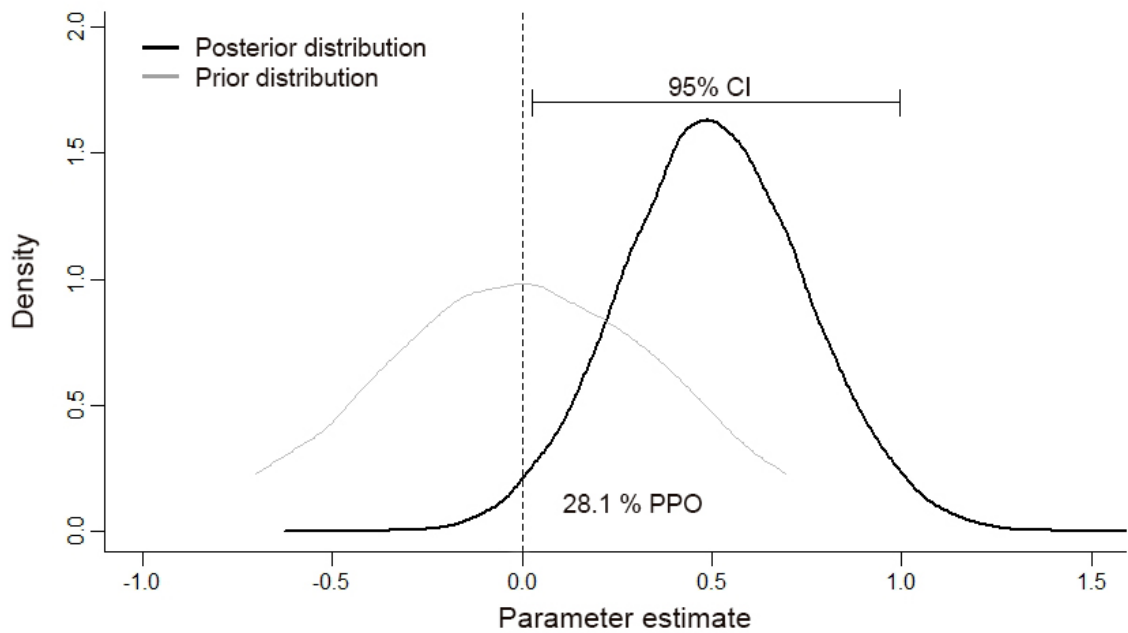


Figure 1.3. Posterior and prior distributions of β_H and amount of prior posterior overlap (PPO). The 95% credible interval (horizontal line) does not overlap with zero (dotted line), providing strong evidence for a nonzero difference in the annual survival probability of adult chickadees between higher and lower elevations.

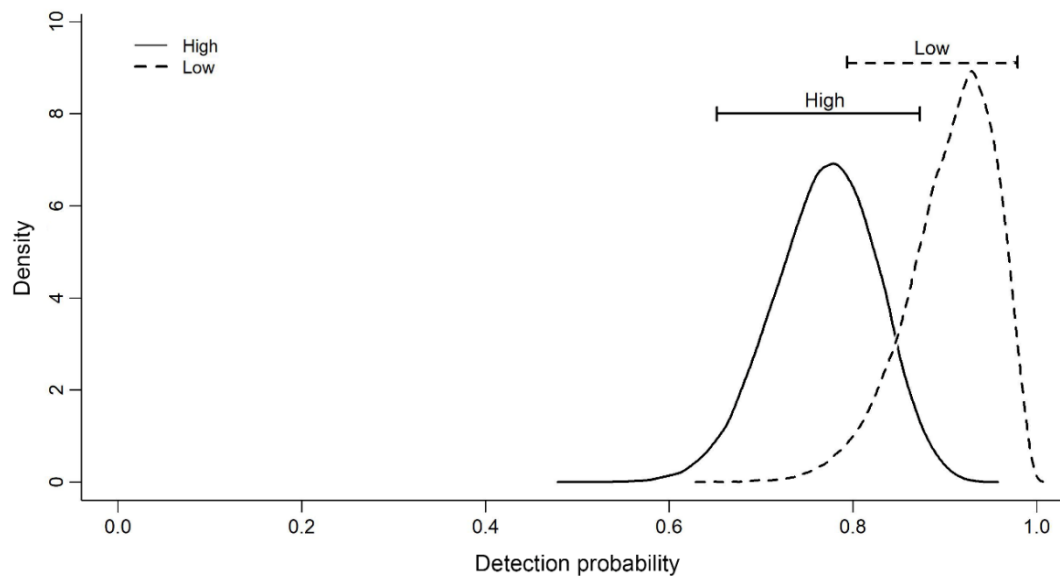
Figure 1.4

Figure 1.4. Posterior distributions of detection probability per site. Horizontal lines indicated 95% credible intervals.

Chapter 2 Variation in Sampling Behavior is Associated with General Rather Than Local Environmental Conditions, Age and Cognitive Abilities

L.M. Benedict^{1*}, A.M. Pitera¹, C.L. Branch^{1,2}, B.R. Sonnenberg¹, V.K. Heinen¹, E.S. Bridge³, V.V. Pravosudov¹.

¹Department of Biology and Ecology, Evolution and Conservation Biology Graduate Program, University of Nevada, Reno, Reno, NV, U.S.A.; ²Cornell Lab of Ornithology, Cornell University, Ithaca, NY, U.S.A.;

³University of Oklahoma, Oklahoma Biological Survey, Norman, OK, U.S.A.

*Corresponding author, Lauren Benedict Nguyen, published as L.M. Benedict

Publication Citation

Benedict, L. M., Pitera, A. M., Branch, C. L., Sonnenberg, B. R., Heinen, V. K., Bridge, E. S., & Pravosudov, V. V. (2021). Information maintenance of food sources is associated with environment, spatial cognition and age in a food-caching bird. *Animal Behaviour*, 182, 153–172. [doi: 10.1016/j.anbehav.2021.10.009](https://doi.org/10.1016/j.anbehav.2021.10.009)

Abstract

Traditionally, exploration and exploitation of resources have been viewed as mutually exclusive behaviors in which animals can either allocate time to gathering information or to using known resources. But these behaviors can also be viewed as opposite ends of a continuum, with intermediate behaviors, such as information maintenance, that balance exploration and exploitation. Updating previously acquired information through information maintenance sampling can allow animals in unpredictable environments to track changing environmental conditions. Theoretical models predict that the degree of involvement in information maintenance should depend on environmental predictability and harshness -- when the overall environment is harsher and less predictable, animals should update previously acquired information more frequently because such information is less certain and could change quickly. We tested this hypothesis by allowing wild food-caching mountain chickadees (*Poecile gambeli*) to visit and sample multiple feeders with temporarily stable, unlimited food for five consecutive winters. We used an index of feeder use breadth to explore how feeder visits across multiple feeders varied with environmental harshness. Each feeder visit is associated with information updating and more information maintenance should be associated with distributing visits across more feeders. While controlling for the total number of visits by each individual, we found that (1) chickadees re-distributed feeder use among more feeders when environmental conditions were harsh and unpredictable, (2) juveniles had a higher feeder use breadth than adults and individuals reduced their feeder use breadth as they aged, (3) better spatial learning and memory ability but not

spatial cognitive flexibility was associated with smaller feeder use breadth and (4) learning associated with decreased food availability reduced subsequent feeder use breadth. Our data supported our predictions that factors affecting the predictability of resource information (environmental conditions and individual characteristics such as cognition and age) affect how individuals engage in information maintenance.

Keywords

Information maintenance; information updating; exploration; exploitation; sampling behavior; environmental predictability; spatial cognition; food-caching species; optimal sampling; chickadee

Introduction

Many animals live in variable and heterogeneous environments: weather conditions, predator densities, food sources and mate quality can all vary with space and time and change rapidly. Such environmental variation creates a constantly shifting information landscape in which an individual's certainty in the state of their surroundings progressively diminishes as information becomes outdated (Dall et al., 2005). The consequences of making decisions based on outdated information could range from mild (e.g., wasted energy foraging) to disastrous (e.g., starvation as a result of poor anticipation of future resource distributions; Shettleworth et al., 1988). However, gathering entirely new information can be costly, as many exploratory behaviors take time and energy that cannot be spent exploiting resources (Berger-Tal et al., 2014). To balance the costs of pure exploration and pure exploitation, animals rely on learning and memory abilities as well as information updating behaviors (Berger-Tal et al., 2014; Stephens and Dunlap, 2017).

Information updating behaviors (also known as information or knowledge maintenance) allow an individual to relearn or update part of their existing knowledge. Within the exploration-exploitation continuum, information updating is an intermediate phase in which individuals both exploit resources and keep a constant, optimal level of up-to-date knowledge through directed learning behaviors, such as sampling (Berger-Tal et al., 2014). Sampling refers to repeated visits or observations that allow an individual to update information (Krebs et al., 1978; Tamm, 1987; Shettleworth et al., 1988; Krebs and Inman, 1992; Dunlap et al., 2017). For example, females often have incomplete

information about the quality of potential mates and will repeatedly visit specific males (i.e., they will sample males) to better assess mate quality before selecting a mate (Luttbeg, 1996). In a foraging context, sampling is often observed when an individual is presented with multiple food sources: first, the animal explores the available sources, then repeatedly visits each source to determine quality and consistency (e.g., Lima, 1984; Dunlap et al., 2017). If an individual stops visiting multiple sources and only forages from one, it has moved from the information updating phase to the pure exploitation phase.

The major conceptual and still debated question is when and how often animals should sample their environment to update previously learned information (Dunlap et al., 2017). Bayesian foraging hypotheses suggest that the decision-making process resembles Bayesian updating (Valone, 2006). Suppose an individual has some previous experience and knowledge of the distribution of resource quality in an area obtained through exploration or knowledge acquisition (the prior distribution). The individual can update specific aspects of this knowledge that may be incomplete or outdated via sampling until it more closely resembles the true distribution of resource quality (posterior distribution). This updated knowledge can then be used to decide whether it is worthwhile to continue foraging in the current area or sample elsewhere (Iwasa et al., 1981; Dall et al., 2005; McNamara et al., 2006; Valone, 2006). The individual should only choose to update that information if the value of the information gained is greater than the cost of sampling to obtain or to maintain the information (Dall et al., 2005). Oversampling could be costly in terms of physical effort and lost opportunities for other activities while under-sampling

could lead to missing critical environmental changes or making poor choices about which resources to prioritize (Shettleworth et al., 1988; Dunlap et al., 2017).

But how do animals assess the value of updated information and determine how often to sample? During the information maintenance phase, continuous updating of previously acquired information is expected to be strongly affected by temporal unpredictability of the environment (Berger-Tal et al., 2014). When the environment is more predictable, there should be less of a need to constantly maintain fully updated information because the individual's prior knowledge is sufficient to predict the environment; the future value of the updated information would be low. On the other hand, when the environment is relatively unpredictable, maintaining existing information would be extremely beneficial and sampling could allow individuals to maintain the most current information about resources to use (Stephens, 1989). Similarly, environmental harshness may also affect the cost-benefit analysis of information maintenance: in harsh environments, the costs of not finding food might be higher due to greater caloric requirements or greater risk of starvation. Animals would be expected to update information more when conditions are harsh, and it is more critical to know where food is available in case one food source fails. However, there is little work testing theoretical predictions about when animals should shift along the exploration, information maintenance and exploitation continuum. Furthermore, environmental predictability consists of multiple components, including food availability and quality, frequency of storms, and weather conditions such as temperature and precipitation patterns. But while some information maintenance studies manipulate environmental predictability via food quality and availability (e.g., Katz and Naug, 2015; Dunlap et al., 2017), few explore how

the overall unpredictability in environmental conditions may affect information updating behaviors and none explore how environmental harshness may play a role.

Here, we tested predictions from a theoretical model (Berger-Tal et al., 2014) that information maintenance sampling is a function of the overall environmental uncertainty and harshness in a wild food-caching bird, the mountain chickadee (*Poecile gambeli*). Testing these predictions in laboratory conditions presents major logistical challenges, as it is difficult to manipulate the prevailing environmental conditions without altering the testing paradigm (i.e., availability and variability of food). We conducted our study in wild birds in their natural environment, keeping the experimental foraging locations temporarily stable while evaluating engagement in information maintenance sampling under different environmental conditions (e.g., climate-related variables). Mountain chickadees are an excellent species to explore information maintenance within a foraging context because they forage for food items one-at-a-time. When presented with a supplemental feeder, they will retrieve one food item for each visit to the feeder before leaving to eat or cache the food in a different location. Thus, each visit is associated with information acquisition, updating and exploitation, especially if visual access to the food within each feeder is only available through visitation, as it is in our system. Each visit to a previously visited feeder (i.e., after birds first discover the feeders through exploration) is then considered as information maintenance sampling because it results in information update about the food status in that feeder.

We predicted that birds should continue visiting multiple previously visited food sources, even when these sources are temporarily stable, to maintain the most updated

information about each in case one fails. More frequent information updating should increase information reliability; the more frequently a bird visits each previously discovered food source, the more certain that bird will be that the food source continues to provide food. Such information maintenance sampling should depend on the overall environmental conditions: if the probability of finding new food sources is low (i.e., in unpredictable environments) or if the consequences of not finding food quickly are high (i.e., starvation in harsh environments; Lima, 1986; Pravosudov and Grubb, 1997; Pitera et al., 2018), then the value of up-to-date information on food sources should be high and birds should engage more in information maintenance (Berger-Tal et al., 2014). Conversely, if the environment is comparatively mild and predictable, the value of constantly updating information will be lower and animals should engage in information maintenance sampling less, as there are fewer missed opportunities to forage (i.e., fewer storms) and such opportunities are less costly (i.e., lower metabolic needs).

Individual characteristics such as cognitive abilities and age-related experience may also affect environmental certainty and hence information updating behavior. Better learning and memory abilities likely increase environmental predictability, as the individual can rely on these abilities to find previously made food caches and to learn and remember more naturally available food sources (Dunlap and Stephens, 2012; Pitera et al., 2018). Food-caching species, in particular, have evolved food-caching behavior as a strategy to compensate for environmental harshness and unpredictability but they rely on spatial cognitive abilities to access their caches (Pravosudov and Roth, 2013). Similarly, age should be associated with more experience and might allow older individuals to fine-tune their responses to environmental cues and better predict changing environmental

conditions. This would likely result in less need to keep updating previously acquired information for older, more experienced individuals.

We tested these hypotheses using five years of data from our long-term field system of food-caching mountain chickadees in the northern Sierra Nevada. Birds at higher elevations in our system experience harsher and less predictable winter conditions and have better spatial cognitive abilities associated with greater reliance on stored food to survive winter than birds at milder, more predictable lower elevations (Freas et al., 2012; Croston et al., 2016, 2017). There is large variation in winter environment across years. In this system, chickadees use multiple supplemental food sources (arranged in feeder arrays) that are temporarily stable (i.e., food quality and availability are constant within the data collection period, but not within the entire season or year). Visitation of multiple feeders within our feeder arrays is highly variable among years and individuals, providing a convenient platform to test the following predictions: (1) individuals should distribute more visits to more feeders in order to maintain the most updated information under more severe winter conditions, whether associated with yearly variation or elevation; (2) adults should distribute visits to fewer feeders than juveniles and individuals should distribute their visits to fewer feeders with increased age as a result of experience; (3) individuals with better spatial cognitive abilities should distribute their visits to fewer feeders than those with worse spatial cognitive abilities (Fig. 2.1).

To estimate breadth of feeder visitation (e.g., relative distribution of visits to multiple feeders) across the eight feeders in a given feeder array, we used a measure of niche breadth (Levins' measure; Levins, 1968). This metric provides an estimate of how

each bird distributes its visits across all available feeders, from visiting one feeder exclusively (i.e., minimum feeder use breadth) to visiting each feeder an equal number of times (i.e., maximum feeder use breadth). Unlike simply recording the number of visits per feeder, which cannot describe the overall relative use of all eight feeders, the Levins measure provides a robust measure of feeder use breadth across all available feeders.

It is important to differentiate between pure exploitation and information maintenance in this system. Exploitation of different feeders could be affected by the social environment (competition) or frequency of visits, as more visits may result in visiting more feeders. Alternatively, birds may visit multiple feeders randomly. Feeders were arranged within a relatively small spatial scale (<2m), so the costs of visiting multiple feeders were potentially low. We predicted that if birds engaged in more information maintenance rather than in pure exploitation, they should not visit all feeders randomly and they should increase or decrease their feeder use breadth based on the environmental predictability (as predicted in Berger-Tal et al., 2014), independently of the social context or the total number of visits.

Theoretical models predict that age associated with life expectancy should affect the value of sampling and information maintenance (Eliassen et al., 2007). According to this hypothesis, older individuals would invest more in exploitation than exploration, as the value of both sampling to acquire new information and to update previously acquired information is less when life expectancy is shorter. We do not think this applies to our system, as the life expectancy of chickadee adults may actually be higher than that of

juveniles (Benedict et al., 2020). In our system, experience associated with age is more likely to affect the exploration-exploitation continuum.

During one year of the study (2019-20), we also explored whether learning associated with experimental manipulations of food availability in different feeders affected feeder use for information updating. We looked at feeder use breadth both before and after spatial cognitive testing, during which food was temporarily restricted at the feeders and individuals had to learn that only one feeder provided food while all other feeders provided no food reward. We predicted that after cognitive testing, when food availability is restored at all feeders, individuals that have previously learned that most feeders were not consistently rewarding could be expected to have reduced feeder use breadth. Specifically, better learners should distribute their visits to fewer feeders, as they have learned that certain feeders have recently not provided food and information gained by sampling these feeders when other feeders provide food should have a lower value. In other words, we predicted individuals should distribute their visits to fewer feeders and have a smaller feeder use breadth in response to this previously learned information, regardless of current feeder status.

Methods

Study Site

The study was conducted at our long-term mountain chickadee study system in Sagehen Experimental Forest (Sagehen Creek Field Station, University of California

Berkeley) in the Sierra Nevada, 10 km north of Truckee, CA, USA (Freas et al., 2012; Croston et al., 2016, 2017; Kozlovsky et al., 2018; Tello-Ramos et al., 2018). Our study system includes two primary areas that we refer to as low (1900m) and high (2400m) elevations. These areas differ in winter environmental harshness – at high elevations winter conditions last longer with lower temperatures, higher and longer-lasting snow cover and more frequent and unpredictable snowfalls compared to lower elevations (Barbour and Minnich, 2000; Kozlovsky et al., 2018). We trapped chickadees annually (2014-2020) using mistnets at multiple established feeders (August-April) and at established nestboxes during the breeding season (May-July). All birds were banded with unique color band combinations, including a passive integrated transponder (PIT)-tag (IB Technology, Leicestershire, UK). Sex was determined by breeding status (i.e., presence of cloacal protuberance for males, brood patch for females, or visual observations of behavior) during breeding season and by wing length (female wing length ≤ 67 mm, male wing length ≥ 72 mm) during the non-breeding season. Age at initial capture (“juvenile” or “hatch year” if less than one year of age, “adult” if at least one year or older) was determined during banding using multiple plumage characteristics (Meigs et al., 1983; Pyle, 1997), breeding status and our records of nestling status at the study site (nestlings banded with metal United States Geological Survey leg bands). Ages at subsequent detections were estimated from previous records.

Environmental harshness was estimated by average temperatures and snow depth (Pitera et al., 2018). Snow depth, a proxy for the harshness and duration of winter snowstorms, additionally provided an estimate of environmental predictability, as snowstorms vary in their harshness and duration, preventing birds from foraging for

unpredictable amounts of time. We obtained climate data from three SNOwpack TELemetry Network (SNOTEL) weather stations (supported by the United States Department of Agriculture's Natural Resources and Conservation USDA Natural Resources Conservation Service, 2020) located within and nearby the study system. High elevation climate data were sourced from #541 – Independence Lake (ca. 2500m). Low elevation climate data were sourced from two stations and averaged: #540 – Independence Camp (ca. 2100m) and #539 – Independence Creek (ca. 1950m). We downloaded daily average temperatures and daily snow depth, using the mean values for each annual sampling period in the analyses. Daily maximum and minimum temperatures were also obtained to characterize the two elevation sites used in the study (Fig. S2.1) but were not used in the final analyses. We only used climate data for the specific periods of testing each year (Table S2.1).

Sampling at Feeder Arrays

Data collection for feeder use behavior.

Beginning in 2015, data on feeder visitation were collected over five winter seasons (2015-2020) using radio frequency identification (RFID)-enabled “smart” feeders (Bridge and Bonter, 2011) that allowed automatic detection of all individuals with PIT-tags. Feeders were arranged in spatial arrays, each consisting of 8 feeders mounted equidistantly to a 122x122 cm frame and raised ca. 3 m above the ground using wire rope and pulley system attached to 4 trees, to avoid damage by squirrels and bears. Four arrays were established at the study area in 2014 (2 arrays per elevation, ca. 1.2 km apart;

Croston et al., 2016). Each feeder was equipped with a battery-powered mechanical door that controlled access to the food (black oil sunflower seeds) via a programmable circuit board. Circuit boards could be programmed to one of three settings (“modes”): (1) “open” mode – feeder doors were always open and any bird could see food inside the feeders; (2) “all” mode – feeder doors were closed so the food inside the feeders was not visible until a PIT-tagged bird landed on the perch (containing an antenna), opening the door and allowing that bird to access the food; (3) “target mode” – feeder doors were closed and would only open for specific PIT-tagged birds, such that each bird could only access food at one feeder at an array to assess spatial learning and memory (Croston et al., 2017). “Open” mode was used to habituate birds to the feeders, “all” mode was used to collect sampling data and “target” mode was used to conduct spatial cognitive tests. During each mode, feeders recorded visitation data (bird ID, date, time) for any PIT-tagged bird that landed on the feeder perch (i.e., a “visit”).

Annual experiments began with a habituation phase, in which feeders were set to “open” mode every fall (Table S2.1) and continued at least 10 days or until a sufficient number of PIT-tagged chickadees were detected foraging from the arrays (ca. 100 per elevation). Then feeders were switched to “all” mode for at least 8 days to assess relative distribution of feeder use (e.g., feeder use breadth) and collect data for information maintenance sampling behavior. A key aspect of this mode was that the food was accessible to every PIT-tagged bird but was not visible until a bird visited the feeder, triggering the door to open and providing access to the food. This meant that birds could only update the food status of each feeder by visiting it allowing the “smart” feeders to record the visit. So, every feeder visit can be considered as information updating. Finally,

after the data collection period for information maintenance sampling, feeders were switched to “target” mode for testing spatial cognitive learning and memory and reversal spatial learning and memory (Croston et al., 2017). During data collection in “all” and “target” mode, great care was taken to ensure that feeders did not malfunction. Food levels were maintained during “all” and “target” mode such that no feeders ran out of seeds during data collection.

Criteria for information maintenance sampling behavior.

A bird was considered to be engaged in information updating “sampling” when it visited feeders in an array while food was accessible but not visible (“all” mode). Birds had to physically visit the feeder to determine whether food was present by triggering the door to open and reveal access to the seeds. Unlike other birds that sit and eat at feeders (e.g., finches), chickadees visit feeders for no more than a few seconds, select one seed per visit and leave the array to eat or cache the seed elsewhere. This results in significantly reduced social interactions at the feeders. Chickadees are the main species using the feeders in the winter. Nuthatches sometimes visit the feeders but only when they are in the “open” mode with seeds clearly visible and accessible. Once feeders are in “all” mode, allowing only PIT-tagged chickadees to access the food, no other species visit the feeders. Given substantial efforts to band as many chickadees as possible, we estimate that most birds near the feeders are PIT-tagged (>90%). The untagged birds stop visiting the feeders during “all” mode, instead foraging on the ground under the feeders.

Feeder sampling metric.

Relative distribution of multiple feeders use or feeder use breadth was estimated using the Levins' measure (Levins, 1968), a measure of niche breadth (\hat{B}) that estimates how uniformly individuals use a given set of resources (also known as the species equivalent of the Simpson's diversity index; Simpson, 1949; Jost, 2006). In this feeder sampling context, feeder use (niche) breadth for each bird was determined from the proportion of visits \hat{p} to each feeder j [Eq. 2.1] at the array.

$$\hat{B} = \frac{1}{\sum \hat{p}_j^2} \quad (\text{Eq. 2.1})$$

Levins' measures of feeder use breadth ranged from $\hat{B} = 1$ (all visits to one feeder) to $\hat{B} = 8$ (visits equally distributed across all 8 feeders at an array); higher values indicated higher feeder use breadth associated with more information updating. This metric was independent of the number of visits by each bird unless the bird made fewer visits than the number of resource states (i.e., fewer than 8 visits per day). Fewer than 8 visits would result in an artificially lowered Levins' measure. As such, we set a minimum threshold of 64 visits for birds to be included in the study, allowing for birds to visit at least 8 times per day for 8 days (Fig. S2.2). This was important because this study aims to explore how birds revisit feeders to update previously learned information. Thus, we needed to be sure that birds had the opportunity to initially explore the 8 feeders before we consider their visits as information maintenance behavior. The majority of the

excluded birds visited fewer than 10 times (see Fig. S2.3A for exact number of all excluded birds by year, elevation and sex and age) and our exclusions were not biased toward any sex or age group (Fig. S2.3B).

To further control for different levels of participation between birds, the Levins' measure was calculated per bird per day and averaged across the number of days visited during each data collection period. The total number of visits by each bird for each annual data collection period was then included as a covariate in all models.

Low elevation data in 2017-18 and 2018-19.

After setting the minimum visit threshold at 64 visits, we implemented two other exclusions. First, if a bird visited two arrays within the same season, data were excluded from the array that had fewer total visits (this affected $N = 13$ birds). Second, no data were used from 2017-18 or 2018-19 at low elevation because few birds visited the feeders before and during the data collection period and visits were inconsistent. This excluded $N = 4$ birds from the dataset that otherwise would have fit our 64 visit criteria.

Post-cognitive testing data collection.

During one year (2019-20), we collected data for information maintenance sampling behavior both before and after conducting spatial cognitive tasks, to evaluate both the effect of learning and memory ability on sampling strategies (when all feeders are stable, equal and have reliably provided food before cognitive testing) as well as the direct effect

of learning new information on sampling (after some feeders stopped providing stable food for a short period during cognitive testing). To do this, the first “pre-cognitive testing” sampling period was collected as described above in 2019-20. Following spatial cognitive testing, feeders were switched from “target” mode to “open” mode for three days, during which feeder doors were open, allowing birds to see food available at all feeders. Then feeders were switched to “all” mode, during which we collected “post-cognitive testing” sampling data for four days following the same protocol for information maintenance data collection (Table S2.1).

Spatial cognitive tests

Two spatial cognitive tasks were conducted annually (Table S2.1). First, individuals were given a spatial learning and memory task in which each bird was assigned to only one rewarding feeder (feeders set to “target” mode, birds assigned to specific feeders by programming “smart” feeders to only open doors for specific PIT-tagged birds). Birds were individually and pseudorandomly assigned to a feeder that they had previously visited infrequently during data collection for sampling behavior. For the second task, birds were given a reversal spatial learning and memory task (a proxy for cognitive flexibility, Croston et al., 2017). Each bird was re-assigned to a new rewarding feeder, separating birds that had previously been assigned to the same rewarding feeder to control for the possibility of social learning (Croston et al., 2017; Tello-Ramos et al., 2018). To assess spatial learning and memory in the first task and cognitive flexibility in the second task, we used the number of unrewarding feeders visited prior to visiting the

rewarding feeder (location errors) during each trial (Croston et al., 2017). Each trial started with a visit to any feeder in the array and ended with a visit to the assigned rewarding feeder. Following our previous work, we used the mean number of location errors per trial during the first 20 trials of each task separately to assess spatial learning and memory ability and cognitive flexibility (Croston et al., 2017; Tello-Ramos et al., 2018; Sonnenberg et al., 2019).

Statistical Methods

To evaluate individual sampling strategies, we fit linear mixed effects models in R (R Core Team, 2018) using the R package *lmerTest* (Kuznetsova et al., 2017). To control for differences in individual participation, the total number of visits per bird per data collection period was included as a fixed effect in all models with mean Levins' measure as the response variable. To evaluate possible social effects on mean Levins' measure due to other conspecifics present at the feeders, models were fit with and without the total number of birds present at each array during the data collection period (Table S2.3). To better understand the factors affecting how many times individuals visited the feeder, additional models were fit with total number of visits per bird per data collection period as the response variable (Figures S5- S11). All linear effects models held individual ID as a random effect. All numeric fixed effects were scaled. We did not test for every possible interaction between predictor variables, preferring to only consider the interactions that we hypothesized might strongly affect sampling behavior.

Fixed effects used in models.

To control for differences in participation among birds, all models controlled for the total number of visits per bird per year (as a fixed effect). We also controlled for the number of days a bird participated (out of 8 total days of each annual data collection period) in several models. To further ensure that our sampling data were minimally affected by social interactions at the feeders, we controlled for the number of birds present at a feeder array.

We used the same combinations of fixed effects to explore variation in Levins' measures and total number of visits for each bird. To evaluate the effects of environmental harshness, we fit two models with the following additional fixed effects: (1) year, elevation, year by elevation interaction; (2) mean snow depth and mean daily temperatures for the period of data collection. Year was not included in models with mean environmental conditions. To evaluate the effect of age and experience, two models were fit with the following fixed effects: (1) year, elevation, age (juvenile vs. adult) and interaction effects of year by elevation, year by age, age by elevation and all three together; and (2) year, number of years sampled (1st, 2nd, etc.; i.e., increasing age) and cohort (first year a bird was observed sampling in our system, e.g., 2015-16, 2016-17). The second model was fit to the subset of data that only included birds that had sampled during at least two years of the study (Table S2.2). To evaluate whether social dominance might explain age-related differences in sampling breadth, we fit a model with year, elevation, sex (male versus female), age (juvenile versus adult) and interaction effects of

year by elevation and age by sex. There is strong evidence in Paridae species for a linear social hierarchy that follows sex and age (adult male > adult female > juvenile male > juvenile female; Dixon 1965; Ekman, 1989, 1990; Gentle and Gosler, 2000) and thus support for an age by sex interaction effect would support social dominance as an explanation for variation in sampling breadth. To evaluate spatial cognition, models were fit with either spatial learning and memory ability or cognitive flexibility (mean errors per trial during the first 20 trials) as fixed effects. Cognition scores were not averaged across years for birds that were observed in multiple years; each year's score was used for that year of sampling data. If a bird did not have both sampling and cognition data for a particular year, those data were excluded from relevant cognition models.

Feeder use after cognitive testing in 2019-20 was evaluated via linear regressions using the *stats* package (R Core Team, 2018). The predictor variables for these linear regressions included total number of visits during the data collection periods for information maintenance sampling (scaled), either spatial or reversal spatial learning and memory ability (scaled) and the total number of visits during the reversal spatial learning and memory task (scaled).

Certain data were not available for every bird; there were individuals for which we did not know sex or were not able to obtain data on cognitive ability. Thus, we ran each model with the maximum number of birds possible (Table S2.2). As models used slightly different datasets, model fit was compared between models using conditional and marginal R^2 values (Nakagawa and Schielzeth, 2013) calculated using the *performance* R package (Ludecke et al., 2020). Within group comparisons were calculated using

estimated marginal means and Tukey post hoc comparisons via *emmeans* (Lenth, 2020). Model assumptions were evaluated by simulating residuals using the R package *DHARMA* (Hartig, 2020). Model output was further evaluated using the *stats* package Type III Analysis of Variance (ANOVA) tests with Satterthwaite's method.

Data were visualized using *ggplot2* (Wickham, 2016) and raincloud plots (Allen et al., 2019). For all boxplots, lower and upper box boundaries represent 25th and 75th percentiles, line inside box is the median, lower and upper error lines indicate 1.5 times greater and lesser than the 25th and 75th percentiles, respectively. Outliers not shown. Transparent points (jittered for clearer viewing) and density plots represent raw data.

Ethics Note

To the best of our knowledge, no birds were harmed by the collection of this data and birds were only handled for a few minutes during banding. We detected no negative effects of using PIT-tags and color bands during our study. The study was approved by the University of Nevada Reno Institutional Animal Care and Use Committee (Protocol 00818, 00046 and 00603) and was in accordance with California Department of Fish and Wildlife Permit D-0011776516-4.

Results

The final dataset used to analyze feeder use behavior included $N = 471$ unique chickadees from two elevations ($N = 261$ at high, $N = 210$ at low; Fig S4). As some birds were recorded in multiple years ($N = 184$), this resulted in 670 total observations. In accordance with our previous work (Croston et al., 2017), birds showed a preference for one feeder that they visited more than all others at both elevations during all five years (high elevation: 0.338 ± 0.05 proportion of total visits; low elevation: 0.329 ± 0.01 proportion of total visits; Fig. 2.2).

Effects of Environmental Harshness

Mean Levins' measure of feeder use breadth varied significantly among years but not between elevations (linear mixed effects model – year: $F_{4,545.58} = 23.85$, $P < 0.001$; elevation: $F_{1,550.38} = 1.36$, $P = 0.24$; Fig. 2.3A). There was a significant year by elevation interaction ($F_{2,625.56} = 7.47$, $P < 0.001$): feeder use breadth was significantly higher at low elevation than at high elevation in one year (2019-20) but did not differ significantly between elevations in the other four years. Feeder use breadth was positively and significantly associated with the total number of visits ($F_{1,644.63} = 67.60$, $P < 0.001$; R^2 conditional = 0.35, R^2 marginal = 0.20). When the total number of birds visiting the arrays was added to the model, the number of birds was not statistically significant (Tables S2.5- S2.6) and did not improve the model fit (R^2 conditional = 0.34). When the number of days each bird participated per year was added to the model, it was not

statistically significant (Table S2.7) and did not improve the model fit (R^2 conditional = 0.35).

Within high elevation, feeder use breadth was significantly smaller in two out of five years (2015-16 and 2017-18) compared to the other three years (Fig. 2.3A). These two years were characterized by much milder winter conditions compared to 2016-17 and 2018-19 which had the record snow cover in the overall region. Within low elevation, feeder use breadth was significantly different among all three years, with the lowest feeder use breadth in 2015-16 (the mildest year) and the highest in 2019-20.

Winter environmental conditions (e.g., temperature and snow depth) varied significantly among the years and hence these conditions were strongly correlated with year (Fig. S2.1), as there was only one data collection period for feeder use breadth per year. Birds' feeder use breadth was significantly higher when mean snow depth was higher and when mean daily temperatures were lower (linear mixed effects model – snow depth: $\beta = 0.21$, $F_{1,528.32} = 29.76$, $P < 0.001$; Fig. 2.3B; mean temperature: $\beta = -0.16$, $F_{1,594.89} = 9.71$, $P = 0.002$; total number of visits: $F_{1,664.97} = 56.40$, $P < 0.001$; R^2 conditional = 0.31, R^2 marginal = 0.14; Fig. 2.3C). When the total number of birds visiting the arrays was added to the model, the total number of birds was not statistically significant (Tables S2.5- S2.6) and did not improve the model fit (R^2 conditional = 0.36).

Age and Sex Differences

The overall effect of age on feeder use breadth was near significance levels, with juveniles having a higher feeder use breadth than adults (linear mixed effects model – age: $F_{1,670.00} = 3.22$, $P = 0.073$; Fig. 2.4). There was significant variation in feeder use breadth among years ($F_{4,638.99} = 20.91$, $P < 0.001$), but no significant differences between elevations ($F_{1,585.16} = 0.07$, $P = 0.80$). Notably, there were significant interactions of year by elevation ($F_{1,663.43} = 3.59$, $P = 0.03$) and age by year ($F_{1,662.89} = 4.19$, $P = 0.002$), but not age by elevation ($F_{1,658.89} = 0.004$, $P = 0.95$) or age by year by elevation ($F_{2,668.49} = 2.73$, $P = 0.07$; total visits: $F_{1,655.52} = 57.19$, $P < 0.001$; R^2 conditional = 0.38, R^2 marginal = 0.23). Post hoc pairwise comparisons (Tukey-adjusted for multiple comparisons) revealed that juveniles had significantly higher feeder use breadth than adults at both elevations during two of the five years (2016-17 and 2019-20), the years which were associated with the most severe winter conditions (Fig. S2.1). When the total number of birds visiting the arrays was added to the model, the number of birds was not statistically significant (Tables S2.5- S2.6) and did not improve the model fit (R^2 conditional = 0.38). When the number of days each bird participated per year was added to the model, it was not statistically significant (Table S2.7) and did not improve the model fit (R^2 conditional = 0.38).

When we included both age and sex in the model, there was no overall significant effect of sex or elevation and the overall effect of age was also not significant (linear mixed effects model – sex: $F_{1,329.71} = 1.15$, $P = 0.28$; elevation: $F_{1,374.96} = 0.81$, $P = 0.37$; age: $F_{1,494.40} = 2.59$, $P = 0.11$). The interaction of age by sex was not statistically

significant ($F_{1,445.64} = 0.13, P = 0.72$), but there was significant variation among years ($F_{4,400.81} = 17.54, P < 0.001$) and a significant year by elevation interaction ($F_{2,456.42} = 6.41, P = 0.002$; total number of visits: $F_{1,481.88} = 31.35, P < 0.001$; R^2 conditional = 0.36, R^2 marginal = 0.20). As sex was not a significant predictor in this model or other models that included an interaction effect of sex by year, it was dropped from following analyses. When the total number of birds visiting the arrays was added to the model, the number of birds was not statistically significant (Tables S2.5- S2.6) and it did not improve the model fit (R^2 conditional = 0.36).

To ensure that this nonsignificant interaction between age and sex was not influenced by excluding birds with too few visits, we also ran the model with all birds that visited more than 8 times per year (the minimum required visits for using the Levins' measure to describe feeder use breadth in this system). However, the interaction between age and sex remained nonsignificant (linear mixed effects model – total visits: $F_{1,559.98} = 49.89, P < 0.001$; year: $F_{4,502.22} = 17.23, P < 0.001$; elevation: $F_{1,433.58} = 0.10, P = 0.75$; sex: $F_{1,378.52} = 0.34, P = 0.56$; age: $F_{1,568.73} = 0.63, P = 0.43$; year by elevation: $F_{3,523.67} = 4.40, P = 0.005$; sex by age: $F_{1,531.88} = 0.02, P = 0.89$; R^2 conditional = 0.41, R^2 marginal = 0.21; $N = 358$).

Increasing Age and Experience

To further explore the association between age and feeder use behavior, we limited the dataset to birds that were recorded repeatedly during multiple years ($N = 184$). Increasing age was estimated by the number of years participated in the study. There was

a significant effect of increasing age on feeder use breadth: chickadees gradually, but significantly reduced their feeder use breadth in subsequent years (linear mixed effects model – increasing age: $\beta = -0.25$, $F_{1,380.89} = 4.77$, $P = 0.03$; Fig. 2.5). There was also significant variation across years (year: $F_{4,312.67} = 7.41$, $P < 0.001$) and the effect of cohort was near significance (cohort: $F_{3,245.79} = 2.62$, $P = 0.051$; total visits: $F_{1,379.84} = 25.04$, $P < 0.001$; R^2 conditional = 0.33, R^2 marginal = 0.18).

Age Classes in the First Year of Feeder Use

To test whether detected differences between adults and juveniles could have been due to specific previous experience with our experimental arrays, we conducted a post hoc analysis in which we used ANOVA to compare the Levins' measure of feeder use breadth between age classes (juvenile vs. adult) within the first year that birds experienced our feeder arrays. We only analyzed the two years which were associated with significant age-class differences in the overall dataset (2016-17 and 2019-20; $N = 230$). Even among the birds that were exposed to our arrays for the first time, there was a significant difference between juveniles and adults ($F_{1,225} = 7.33$, $P = 0.007$) as well as a significant difference between elevations ($F_{1,225} = 5.32$, $P = 0.02$), a nonsignificant difference between years ($F_{3,225} = 0.08$, $P = 0.8$), and a significant interaction between year and elevation ($F_{1,225} = 6.41$, $P = 0.01$).

Spatial Cognition and Cognitive Flexibility

The dataset was limited to the subset of birds that sampled and participated in subsequent cognitive tests in at least one year ($N = 64$ birds excluded with no spatial cognition data, Table S2.2). In a model with age, year, elevation and spatial learning and memory ability, juveniles had significantly higher feeder use breadth than adults in 2016-17 and 2019-20 (linear mixed effects model – age: $F_{1,533.21} = 3.02$, $P = 0.083$; age by year: $F_{4,529.03} = 5.95$, $P < 0.001$) and birds with better spatial learning and memory abilities had significantly smaller feeder use breadth than birds with worse spatial learning and memory abilities regardless of elevation (spatial cognition: $\beta = 0.13$, $F_{1,533.96} = 8.28$, $P = 0.004$; year: $F_{4,512.69} = 17.25$, $P < 0.001$; elevation: $F_{1,465.41} = 0.06$, $P = 0.80$; year by elevation: $F_{2,517.83} = 5.78$, $P = 0.003$; total visits: $F_{1,524.16} = 36.15$, $P < 0.001$; R^2 conditional = 0.32, R^2 marginal = 0.24; Fig. 2.6). When the total number of birds visiting the arrays was added to the model, the total number of birds was not statistically significant (Table S2.5-2.6) and did not improve the model fit (R^2 conditional = 0.28). Also, the interaction between age and cognition was not significant ($F_{1,531.39} = 0.90$, $P = 0.34$;) and it was excluded from the analyses.

Spatial learning and memory abilities remained statistically significant in the model when we used mean snow depth and mean daily temperature during the period of data collection on sampling behavior instead of year and elevation (linear mixed effects model – spatial cognition: $\beta = 0.14$, $F_{1,543.85} = 10.14$, $P = 0.002$; snow depth: $\beta = 0.20$, $F_{1,461.02} = 18.88$, $P < 0.001$; mean temperature: $\beta = -0.20$, $F_{2,529.58} = 10.93$, $P = 0.001$; total visits: $F_{1,540.81} = 39.94$, $P < 0.001$; R^2 conditional = 0.25, R^2 marginal = 0.15).

Additional birds were excluded for reversal spatial learning and memory (cognitive flexibility) analyses (N = 95 birds did not participate in reversal learning tests directly after the first learning and memory task in the same year; Table S2.2). Performance on the reversal spatial learning and memory task was not a significant predictor of feeder use breadth (reversal learning and memory: $\beta = 0.04$, $F_{1,396.88} = 0.54$, $P = 0.46$; year: $F_{4,341.47} = 10.83$, $P < 0.001$; elevation: $F_{1,370.04} = 1.31$, $P = 0.25$; year by elevation: $F_{2,381.67} = 7.08$, $P < 0.001$; total visits: $F_{1,395.39} = 33.49$, $P < 0.001$; R^2 conditional = 0.35, R^2 marginal = 0.21). When the total number of birds visiting the arrays was added to the model, the number of birds was not statistically significant (Tables S2.5- S2.6) and it did not substantially improve the model fit (R^2 conditional = 0.36).

Effect of Experimental Manipulation of Access to Food (2019-20)

In 2019-20, data on feeder use behavior were collected both before and after spatial cognitive testing. Analyses of pre-and post-cognitive testing feeder use data were limited to the subset of birds that participated during both data collection periods and participated in both of the spatial cognitive tasks in 2019-20 (N = 156).

During the regular sampling experiment before cognitive testing, feeder use breadth did not vary significantly with individual's reversal spatial learning and memory ability (linear regression – reversal learning and memory: $\beta = 0.10$, $F_{1,152} = 1.52$, $P = 0.22$; total visits: $\beta = 0.35$, $F_{1,152} = 17.52$, $P < 0.001$; elevation: $F_{1,152} = 6.55$, $P = 0.01$; multiple $R^2 = 0.12$, adjusted $R^2 = 0.11$; $P < 0.001$, Fig. 2.7).

After the cognitive testing period, there was no significant effect of spatial learning and memory ability (from the first spatial cognitive task) on feeder use breadth (linear regression – spatial learning and memory: $\beta = 0.18$, $F_{1,152} = 2.82$, $P = 0.09$; total visits: $\beta = 0.21$, $F_{1,152} = 6.74$, $P = 0.01$; elevation: $F_{1,152} = 0.001$, $P = 0.98$; multiple $R^2 = 0.06$, adjusted $R^2 = 0.04$). However, after cognitive testing, individuals that performed better (made fewer errors) in the reversal spatial learning and memory task had significantly smaller feeder use breadth compared to individuals that performed worse (linear regression – reversal learning and memory: $\beta = 0.26$, $F_{1,151} = 8.33$, $P = 0.004$; total visits: $\beta = 0.29$, $F_{1,151} = 5.92$, $P = 0.02$; elevation: $F_{1,151} = 0.54$, $P = 0.46$; visits during reversal task: $\beta = -0.03$, $F_{1,151} = 0.07$, $P = 0.8$; multiple $R^2 = 0.10$, adjusted $R^2 = 0.07$).

Discussion

Overall, our findings from five years of data generally supported our predictions: chickadees distributed visits more broadly across multiple feeders when the environment was harsher and more unpredictable; juveniles had higher feeder use breadth than adults during the harshest two years of the study; and birds with better spatial learning and memory abilities had smaller feeder use breadths than birds with worse abilities. In addition, feeder use breadth was reduced significantly with age, likely due to increased experience. Chickadees had a preferred feeder that they foraged from more than the others, and yet continued to use other feeders. Our results support the hypothesis that when birds experienced a harsher and less predictable environment, they invested more in

maintenance of previously learned information by updating such information more frequently. Having the most updated information may be beneficial in case previously available food sources become unavailable, so birds would be able to quickly find an alternative source.

Our data suggest that changes in feeder use breadth are associated with environmental conditions and the need to keep updating information rather than with changes in frequency of visitation due to variable energetic needs or potential social factors (e.g., competition for access to the feeders). We think that our data strongly argue against these alternative explanations. First, we found the results discussed above while controlling for variation in the total number of visits per bird in all of our statistical analyses for feeder use breadth and so our results were independent of the total number of visits. Second, if social competition affected our results, we would expect to find differences in feeder use breadth and total number of visits based on social dominance structure; yet there were no differences between males and females and no significant interaction between age and sex, even though females are socially subordinate to males (Dixon 1965, Ekman 1989, 1990; Gentle & Gosler 2001). Third, and most importantly, when the total number of birds present at each array during data collection was added to our models, it was not statistically significant and did not improve model fits (Table S2.6), suggesting that our results were not dependent on variation in social competition. Overall, our data clearly showed that birds did not randomly distribute their visits among the available feeders (the predicted outcome if there were no benefits to changing the feeder use breadth) nor that the birds exhibited any consistent distribution of feeder use

(which could be potentially explained by particular permanent environmental features near the arrays).

The data supported our prediction that harsher and more unpredictable environments overall should be associated with a greater feeder use breadth even when all available food sources are temporarily stable. Although we did not detect predicted elevational differences in feeder use breadth across all years, we found that chickadees distributed visits across significantly more feeders during years with harsher winter conditions, and when it was colder and there was more snow regardless of elevation. This supports the hypothesis that the value of information maintenance is greater in harsh and unpredictable environments, potentially due to higher costs of searching for food in such conditions (e.g., during unpredictable winter storms characteristic to the Sierra Nevada) and because updated information is more reliable when the overall environment is less predictable.

Juvenile chickadees had significantly greater feeder use breadth compared to adults during the two years with the harshest, most unpredictable winter conditions. One potential explanation is that juveniles are socially subordinate to adults, resulting in a more unpredictable environment for younger birds if foraging opportunities at the feeder arrays are limited by losing competitive interactions with more dominant birds. Thus, low social status could be expected to drive juvenile chickadees to engage in more information updating compared to higher status adults, to compensate for the increased unpredictability from social interactions with higher status individuals. This effect might be more observable during harsher years when energetic requirements and competition

over resources might both be increased. However, if dominance interactions were driving variation in feeder use breadth, we would expect to see an interaction between sex and age that represents the linear dominance hierarchy typically shown in Paridae species (adult males > juvenile males > adult females > juvenile females; Dixon 1965; Ekman 1989, 1990; Gentle and Gosler 2000). Yet, we did not detect significant effects of sex or sex by age interaction, suggesting that juvenile females distributed their visits among multiple feeders similarly to juvenile males, in contrast to the social status explanation.

Another possible explanation for greater feeder use breadth in juveniles compared to adults in the two harshest years is that juveniles are inexperienced during their first winter and as a result overcompensate in their information updating behavior. As animals age, they gain experience allowing them to better assess conditions and fine-tune their response to environmental cues by minimizing costs associated with over-engaging in information maintenance (Dall et al., 2005; McNamara et al., 2006). Our results show evidence for this explanation in two indirect ways: (1) juveniles distributed their visits across more feeders than adults in two of the harshest years in the study; and (2) chickadees reduced feeder use breadth (associated with less information updating) as they aged across the five years of our study. Further, Eliassen et al., (2007) suggested that changes in life expectancy should affect information updating behavior and shorter life expectancy should be associated with less information updating as animals do not have time to obtain the benefits. In this case, age may have an effect on allocation of time between exploration, information maintenance and exploitation directly, regardless of any previous experience. We think this explanation is unlikely in our case as all birds

have relatively long life expectancy, which should benefit from more frequent information updating.

However, the effect of age could either be due to age-related experience or to experience specifically with our feeder system. We think it is more likely that this effect is the result of age-related experience for two reasons. First, the difference in feeder use breadth between adults and juveniles remained even when we looked only at birds during their first exposure to the feeders. As birds in both age-classes would have had the same experience with the feeders at that point, these results suggest that some other form of experience (perhaps age-related) or characteristic was driving the observed age effect. Second, the feeders were only consistent and predictable during the data collection for this study-- throughout the rest of the year, the feeder arrays were at times empty (summer), partially full (i.e., only 2 of 8 feeders filled, early fall), refilled infrequently (i.e., some feeders ran out while others remained full, early fall), full with complete visual access to the food (“open” mode before the experiment), or set to “target” mode during cognitive tests (i.e., each bird can only access food at one feeder). Given the uncertain status of the feeders across an entire year, it would be highly unlikely that the bird would learn that the data collection period for this study provides consistent food every year. Finally, we previously showed that performance in cognitive tasks is not affected by the previous experience with arrays (Sonnenberg et al., 2019).

Our results supported the prediction that cognitive abilities are associated with environmental predictability: individuals with better spatial learning and memory abilities engaged less in information updating, as indicated by smaller feeder use breadth, than

birds with worse abilities even when accounting for annual variation and age effects. We have previously shown that birds do not improve spatial cognitive ability with experience -- suggesting that the cognitive effect is not driven by specific experience with our feeder setup (Tello-Ramos et al., 2018, Sonnenberg et al., 2019). In food-caching animals, spatial cognition is needed to retrieve stored food items, decreasing environmental unpredictability by guaranteeing food supply during food scarce seasons. So, in this case, better spatial learning and memory, but not reversal learning ability, is likely to result in more predictable environment as previously made food caches provide a reliable food source. Accordingly, there were no significant associations between spatial cognitive flexibility (i.e., reversal spatial learning and memory ability) and feeder use breadth across the five years of the study. Cognitive flexibility may reflect the ability to track continuously changing information (Tello-Ramos et al., 2018), but it does not seem to be associated with retrieval of food caches in our system (Sonnenberg et al., 2019). Moreover, the ability to better remember more food caches appears to be associated with *reduced* spatial flexibility (Croston et al., 2018). We do not think that the main reason birds with better spatial cognition visit fewer feeders is that these birds remember the last feeder they visited better compared to individuals with worse abilities. If that were the case, cognition would be the only predictor of feeder use breadth; yet both environmental conditions and age affect feeder use breadth independently of cognition. In addition, we only detected the effect of spatial learning and memory but not spatial reversal learning, which also argues against this explanation. More research into spatial cognitive flexibility would be necessary to explore how this ability may relate to information updating behaviors.

Finally, we showed that birds changed their relative use of all available feeders in response to learning information about these feeders. When we compared feeder use breadth in the same birds before and after cognitive testing (during which food was limited at certain feeders), we found that birds that had performed better on the reversal spatial learning and memory task (directly preceding) had significantly smaller feeder use breadth during the post-cognitive testing data collection period than birds that performed worse on the task. This is particularly interesting considering that before cognitive testing, feeder use breadth was associated with spatial learning and memory ability, but not with cognitive flexibility. We argue these results make a distinction between the effects of spatial cognitive ability and the direct effect of learned information about food status in different feeders. While spatial learning and memory ability affected feeder use breadth before food availability changed, information learned during the reversal spatial task affected relative use of feeders afterwards. As birds learned the reversal task, they learned both (a) which feeder was rewarding and (b) which feeders did not provide food. These results are consistent with our predictions: when birds learned that some of the previously rewarding feeders no longer provided food, the value of continuing to visit those feeders was lower and thus they reduced or stopped updating information in those feeders. We do not think that this reduced feeder use was only due to the strong learned association with the rewarding feeder, but that was equally related to learning location of feeders that no longer provided food. Moreover, birds that did not learn which feeders no longer provided food as quickly as other birds continued re-visiting more feeders. These birds were still worse learners before and after spatial cognitive testing; their individual

abilities did not change due to the cognitive testing, just the way they allocated their visits among available feeders.

In conclusion, our study provided support to our hypothesis that animals should engage more in maintenance and updating of previously learned information when overall environmental conditions are harsh and unpredictable. Having the most recently updated information about food sources makes the environment more reliable and should increase the predictability of available food sources. Spatial cognitive abilities and age-related experience, which can reduce an individual's environmental unpredictability, also affected animals' decisions to engage in information updating, further supporting our hypothesis.

Acknowledgements

Authors were supported by the National Science Foundation grants IOS1856181 and IOS1351295 to V.V.P. and DBI 1556313 to E.S.B.; C.L.B. was supported by the NSF DDIG 1600845. AMP was supported by two University of Nevada Reno (UNR) Graduate Student Association grants to support data collection. We thank Rebecca Croston, Dovid Kozlovsky, and Maria Tello-Ramos, who also participated in data collection. Thank you to the staff of Sagehen Creek Field Station (University of California Berkeley) for assistance at our field site. We also thank three anonymous reviewers for constructive criticisms and suggestions that significantly improved the manuscript.

Data Accessibility

Original data and associated scripts are publicly available from Mendeley Data (<https://doi.org/10.17632/csv8nvy7dr.1>). Dryad. SNOTEL climate data publicly available through the United States Department of Agriculture, Natural Resources Conservation Service, National Water and Climate Center: <https://www.wcc.nrcs.usda.gov/snow/>

References

- Allen, M., Poggiali, D., Whitaker, K., Rhys Marshall, T., & Kievit, R.A. (2019). Raincloud plots: a multi-platform tool for robust data visualization. *Wellcome Open Research*, 4:63. <https://doi.org/10.12688/wellcomeopenres.15191.1>
- Barbour, M.G., & Minnich, R.A. (2000). Californian upland forests and woodlands, in: Barbour, M.G., Minnich, R.A. (Eds.), *North American Terrestrial Vegetation*. Cambridge University Press, New York, NY, pp. 161–202.
- Benedict, L.M., Branch, C.L., Pitera, A.M., Sonnenberg, B.R., & Pravosudov, V.V. (2020). Elevation-related differences in annual survival of adult food-caching mountain chickadees are consistent with natural selection on spatial cognition. *Behavioral Ecology and Sociobiology*, 74:40.
- Berger-Tal, O., Nathan, J., Meron, E., & Saltz, D. (2014). The Exploration-Exploitation Dilemma: A Multidisciplinary Framework. *PLoS ONE*, 9(4): e95693. doi:10.1371/journal.pone.0095693
- Bridge, E.S., & Bonter, D.N. (2011). A low-cost radio frequency identification device for ornithological research. *Journal of Field Ornithology*, 82, 52–59. <https://doi.org/10.1111/j.1557-9263.2010.00307.x>
- Croston, R., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., Bridge, E.S., Parchman, T., & Pravosudov, V.V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123, 139–149. <https://doi.org/10.1016/j.anbehav.2016.10.004>
- Croston, R., Kozlovsky, D.Y., Branch, C.L., Parchman, T.L., Bridge, E.S., & Pravosudov, V.V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behavior*, 111, 225–234. <https://doi.org/10.1016/j.anbehav.2015.10.015>
- Dall, S.R.X., Giraldeau, L.-A., Olsson, O., McNamara, J.M., & Stephens, D.W. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology and Evolution*, 20, 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>
- Dixon, K.L. (1965). Dominance-subordination relationships in mountain chickadees. *The Condor*, 64(4), 291–299. <https://doi.org/10.2307/1365577>
- Dunlap, A.S., Papaj, D., & Dornhaus, A. (2017). Sampling and tracking a changing environment: Persistence and reward in the foraging decisions of bumblebees. *Interface Focus* 7, 20160149. <https://doi.org/10.1098/rsfs.2016.0149>

- Dunlap, A.S., & Stephens, D.W. (2012). Tracking a changing environment: optimal sampling, adaptive memory and overnight effects. *Behavioral Processes* 89, 86–94. <https://doi.org/10.1016/j.beproc.2011.10.005>
- Ekman, J. (1989). Ecology of non-breeding social systems of Parus. *Wilson Bulletin*, 101, 263–288.
- Ekman, J. (1990). Alliances in winter flocks of willow tits; effects of rank on survival and reproductive success in male-female associations. *Behavioral Ecology and Sociobiology*, 26, 239-245.
- Eliassen, S., Jørgensen, C., Mangel, M., & Giske, J. (2007). Exploration or exploitation: life expectancy changes the value of learning in foraging strategies. *Oikos*, 116(3), 513-523.
- Freas, C.A., LaDage, L.D., Roth, T.C., II, Pravosudov, V.V. (2012). Elevation-related differences in memory and the hippocampus in mountain chickadees, *Poecile gambeli*. *Animal Behaviour*, 84, 121–127. <https://doi.org/10.1016/j.anbehav.2012.04.018>
- Gentle, L.K., & Gosler, A.G. (2000). Fat reserves and perceived predation risk in the great tit, *Parus major*. *Proceedings of the Royal Society B* 268, 487–491.
- Hartig, F. (2020). DHARMA: residual diagnostics for hierarchical (multi-level / mixed) regression models. (Version 0.2.7).
- Iwasa, Y., Higashi, M., & Yamamura, N. (1981). Prey distribution as a factor determining the choice of optimal foraging strategy. *The American Naturalist*, 117, 710–723.
- Jost, L. (2006). Entropy and diversity. *Oikos* 113, 363–375.
- Katz, K., & Naug, D. (2015). Energetic state regulates the exploration-exploitation trade-off in honeybees. *Behavioral Ecology*, 26(4), 1045-1050. <https://doi.org/10.1093/beheco/arv045>
- Kozlovsky, D.Y., Branch, C.L., Pitera, A.M., & Pravosudov, V.V. (2018). Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *Royal Society Open Science*, 5, 171604. <https://doi.org/10.1098/rsos.171604>
- Krebs, J.R., & Inman, A.J. (1992). Learning and foraging: individuals, groups, and populations. *The American Naturalist*, 140, S63–S84.
- Krebs, J.R., Kacelnik, A., & Taylor, P. (1978). Test of optimal sampling by foraging great tits. *Nature*, 275, 27–31. <https://doi.org/10.1038/275027a0>

- Kuznetsova, A., Brockhoff, P.B., & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. (Version 3.1.2). *Journal of Statistical Software*, 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>
- Lenth, R. (2020). emmeans: estimated marginal means, aka least-squares means (Version 1.4.7).
- Levins, R. (1968). *Evolution in changing environments: One theoretical explorations*. Princeton University Press, NJ.
- Lima, S.L. (1986). Predation Risk and Unpredictable Feeding Conditions: Determinants of Body Mass in Birds. *Ecology*, 67, 377–385. <https://doi.org/10.2307/1938580>
- Ludecke, D., Makowski, D., & Waggoner, P. (2020). performance: assessment of regression models performance (Version 0.4.4).
- Luttbeg, B. (1996). A comparative bayes tactic for mate assessment and choice. *Behavioral Ecology*, 7, 451–460. <https://doi.org/10.1093/beheco/7.4.451>
- McNamara, J.M., Green, R.F., & Olsson, O. (2006). Bayes’ theorem and its applications in animal behaviour. *Oikos*, 112, 243–251. <https://doi.org/10.1111/j.0030-1299.2006.14228.x>
- Meigs, J.B., Smith, D.C., & Van Buskirk, J. (1983). Age determination of black-capped chickadees. *Journal of Field Ornithology*, 54, 283–286.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Pitera, A.M., Branch, C.L., Bridge, E.S., Pravosudov, V.V. (2018). Daily foraging routines in food-caching mountain chickadees are associated with variation in environmental harshness. *Animal Behavior*, 143, 93–104. <https://doi.org/10.1016/j.anbehav.2018.07.011>
- Pravosudov, V.V., & Grubb, T.C. (1997). Energy management in passerine birds during the nonbreeding season, in: Nolan, V., Ketterson, E.D., Thompson, C.F. (Eds.), *Current Ornithology*. Springer US, Boston, MA, pp. 189–234. https://doi.org/10.1007/978-1-4757-9915-6_5
- Pravosudov, V.V., & Roth, T.C., II. (2013). Cognitive ecology of food hoarding: the evolution of spatial memory and the hippocampus. *Annual Review of Ecology, Evolution, and Systematics*, 44, 173–93. <https://doi.org/10.1146/annurev-ecolsys-110512-135904>
- Pyle, P. (1997). Molt limits in North American passerines. *Natural American Bird Bander*, 22, 49–89.

- R Core Team (2018). *R: a language and environment for statistical computing (Version 3.5.2)*. R Foundation for Statistical Computing, Vienna, Austria.
- Shettleworth, S.J., Krebs, J.R., Stephens, D.W., & Gibbon, J. (1988). Tracking a fluctuating environment: a study of sampling. *Animal Behaviour*, 36, 87–105. [https://doi.org/10.1016/S0003-3472\(88\)80252-5](https://doi.org/10.1016/S0003-3472(88)80252-5)
- Simpson, E.H. (1949). Measurement of diversity. *Nature*, 163, 688.
- Sonnenberg, B.R., Branch, C.L., Pitera, A.M., Bridge, E.S., & Pravosudov, V.V. (2019). Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, 29, 670–676. <https://doi.org/10.1016/j.cub.2019.01.006>
- Stephens, D.W. (1989). Variance and the value of information. *The American Naturalist*, 134, 128–140.
- Stephens, D.W., & Dunlap, A.S. (2017). Foraging, in: Menzel, R. (Ed.), *Learning and Memory: A Comprehensive Reference*. Academic Press, Oxford, pp. 237–253.
- Tamm, S. (1987). Tracking varying environments: sampling by hummingbirds. *Animal Behaviour*, 35, 1725–1734. [https://doi.org/10.1016/S0003-3472\(87\)80065-9](https://doi.org/10.1016/S0003-3472(87)80065-9)
- Tello-Ramos, M., Branch, C.L., Kozlovsky, D.Y., Pitera, A.M., & Pravosudov, V.V. (2018). Spatial memory and cognitive flexibility trade-offs: to be or not to be flexible, that is the question. *Animal Behaviour*, 129–136. <https://doi.org/10.1016/j.anbehav.2018.02.019>
- [dataset] USDA Natural Resources Conservation Service. (2020). *SNOwpack TELEmetry Network (SNOTEL)*. Ag Data Commons. <https://data.nal.usda.gov/dataset/snowpack-telemetry-network-snotel>. WWW Document. Accessed 2021-01-21. <https://data.nal.usda.gov/dataset/snowpack-telemetry-network-snotel>. (accessed 7.15.20).
- Valone, T. J. (2006). Are animals capable of Bayesian updating? An empirical review. *Oikos* 112: 252-259.
- Wickham, H. (2016). *ggplot2: elegant graphics for data analysis (Version 3.2.1)*. Springer-Verlag, New York.

Figures

Figure 2.1

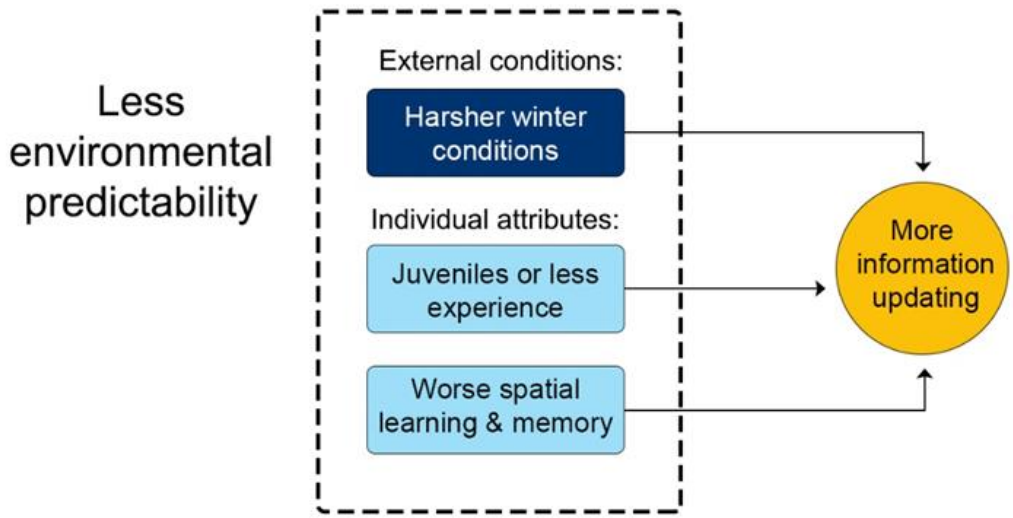


Figure 2.1. Predictions. Individual attributes (light blue) and environmental factors (dark blue) lead to engagement in more information maintenance/updating sampling (arrows) because they affect how predictable the environment is for each individual.

Figure 2.2

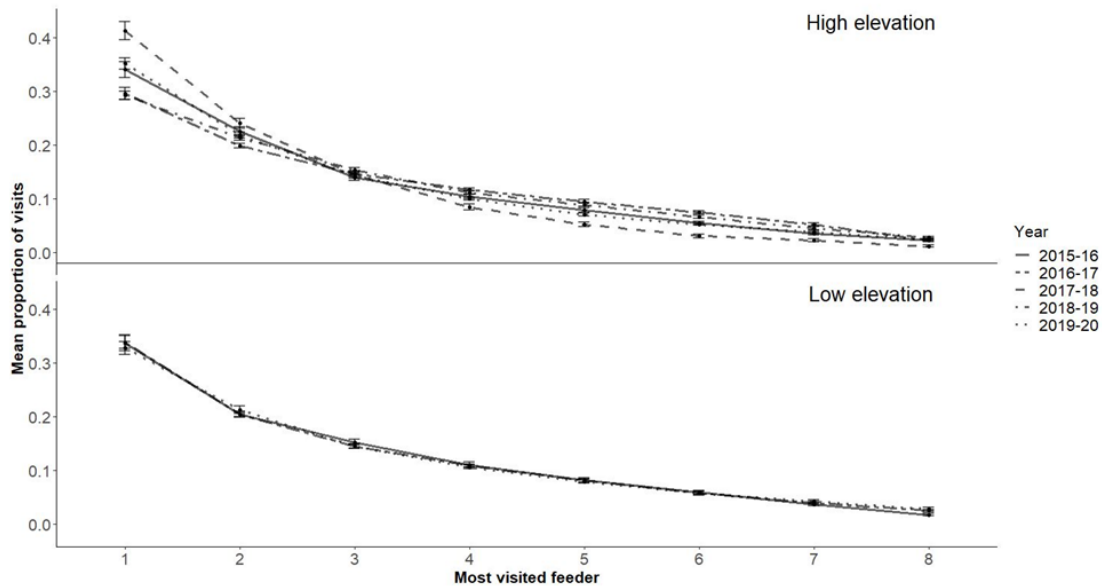


Figure 2.2. Mean proportion of visits per feeder, ordered from most visited to least visited for each bird (1-8) during the 5 years of study. Error bars indicated standard errors per feeder. $N = 471$.

Figure 2.3

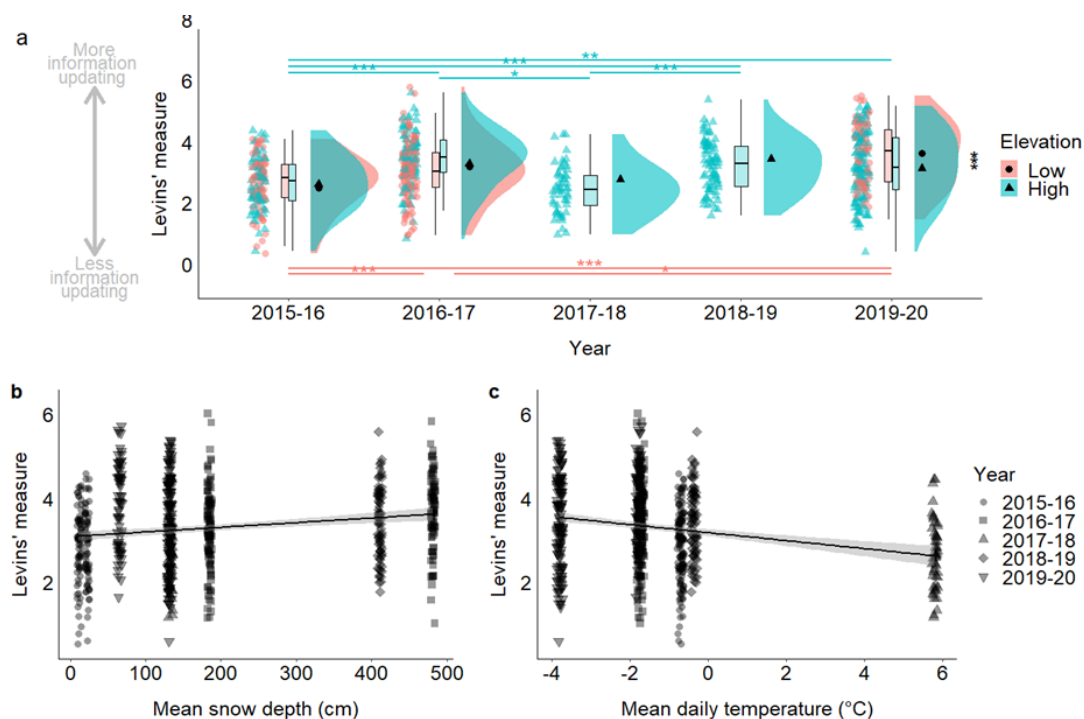


Figure 2.3. Levins' measure of feeder use breadth during the 5 years of study (A) by year at high and low elevations (blue and pink respectively), depicted with estimated marginal means from linear mixed effects model (black shapes); (B) by mean snow depth across both elevations; and (C) by mean daily temperature across both elevations. Significance levels from Tukey-adjusted post hoc pairwise comparisons indicated in panel A by stars ('*' <math><.05</math>, '**' <math><.01</math>, '***' <math><.001</math>) for within-elevation differences between years (lines; pink lines above plot for high elevation comparisons and blue lines below plot for low elevation comparisons) and within-year differences between sites (black stars). Linear regression lines shown in panels B and C by black lines with grey shaded 95% confidence intervals. $N= 471$.

Figure 2.4

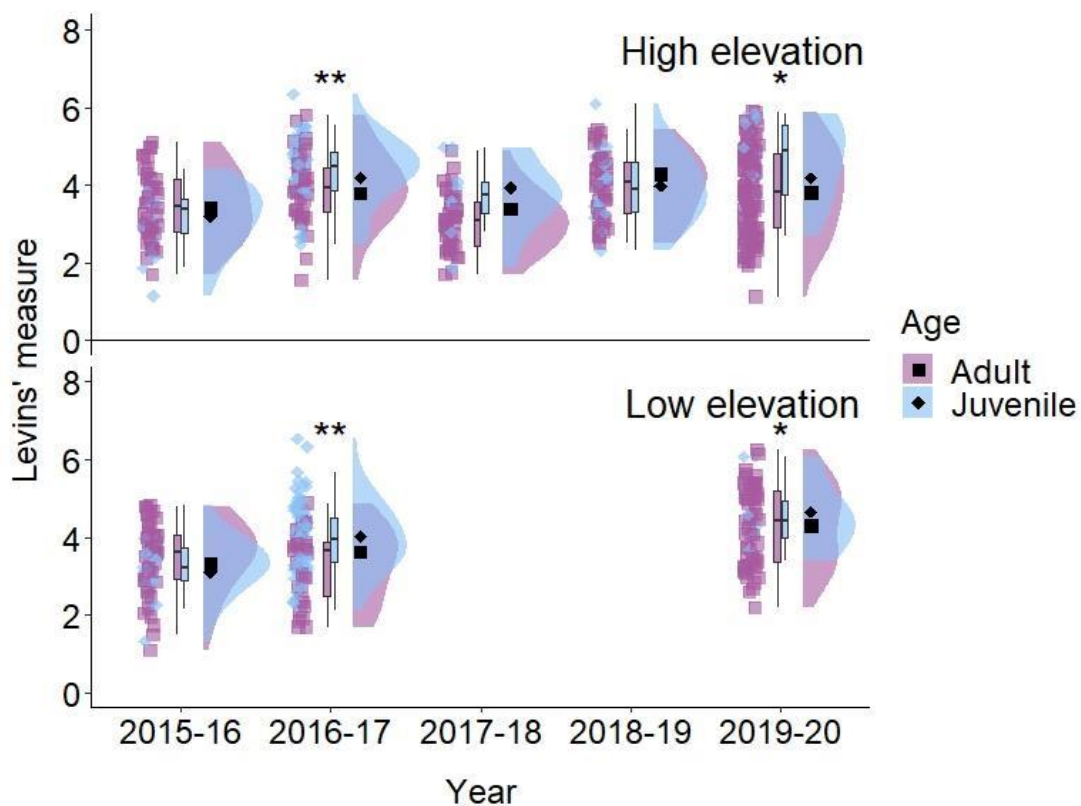


Figure 2.4. Levins' measure of feeder use breadth by age, year and elevation with estimated marginal means from linear mixed effects model (black shapes). Significance levels indicated by stars (*' < .05, '**' < .01, '***' < .001). $N = 471$.

Figure 2.5

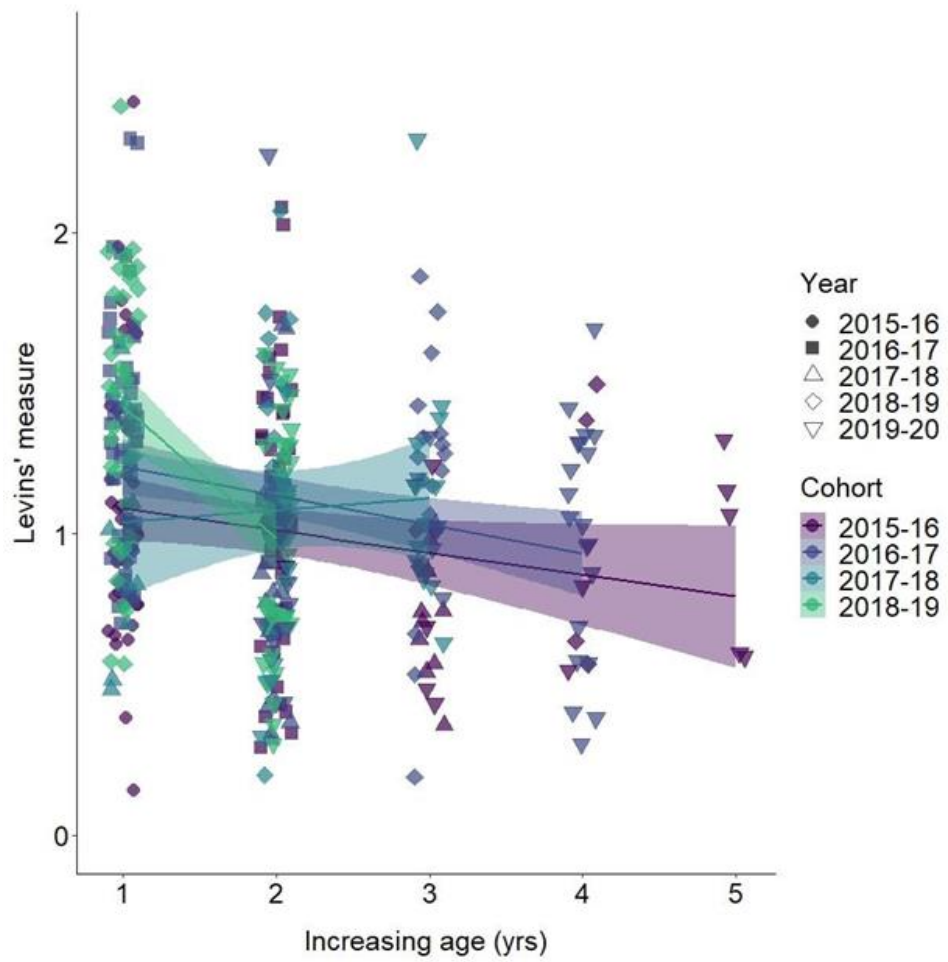


Figure 2.5. Effect of increasing age (i.e., number of years , 1st, 2nd, etc.) on mean daily Levins' measure of feeder use breadth. Lines represent simple linear regressions with 95% confidence intervals (shaded). Cohorts group birds according to the first year recorded sampling. *N*= 184.

Figure 2.6

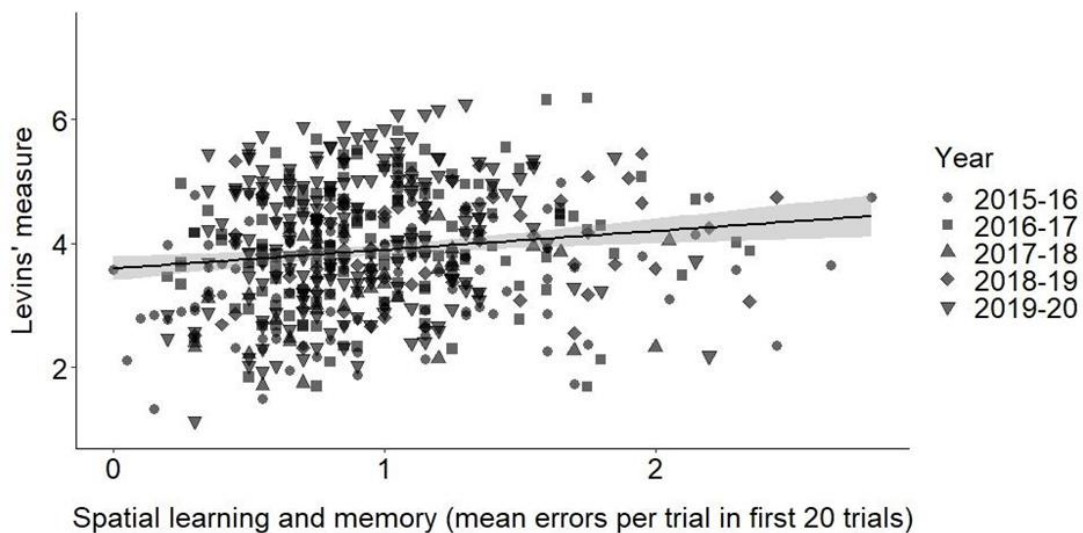


Figure 2.6. Levins' measure of feeder use breadth and spatial learning and memory ability (mean number of errors per trial in the first 20 trials) for all years of study (2015-20). Linear regression lines with 95% confidence intervals (grey). Birds that made fewer errors, indicating better spatial learning and memory ability, sampled less than birds that made more errors. $N = 411$.

Figure 2.7

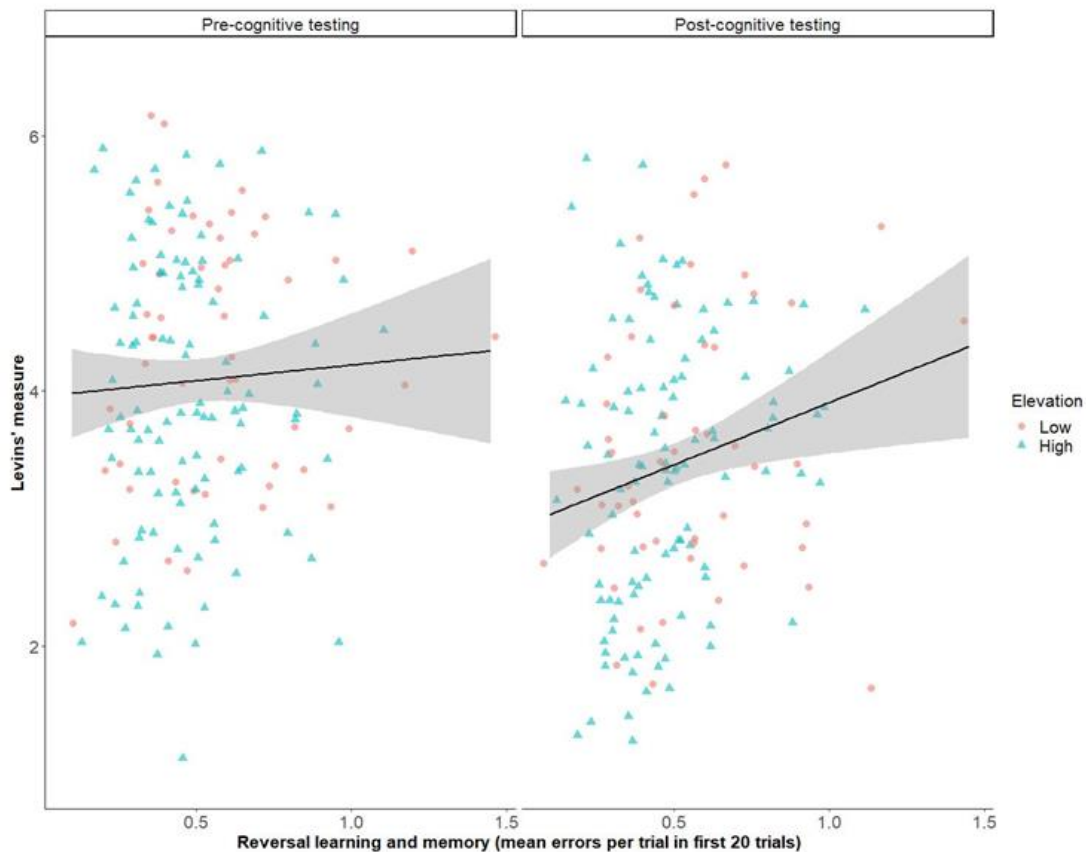


Figure 2.7. Levins' measure of feeder use breadth and reversal spatial learning and memory ability (mean errors per trial in first 20 trials of cognitive task) before and after cognitive testing in 2019-20. Linear regression lines with 95% confidence intervals (grey). During the post-cognitive testing period, birds that made fewer errors, indicating better reversal learning and memory ability, sampled less than birds that made more errors. $N = 157$.

Supplement

Supplemental Tables

Table S2.1: Data collection periods

Year	Elevation	Pre-sampling period ('open' mode)	Data collection period for sampling ('all' mode)	Post-cognitive testing ('all' mode)
2015-16	High	4-Nov-2015 – 11-Nov-2015	11-Nov-2015 – 18-Nov-2015	NA
2015-16	Low	4-Nov-2015 – 11-Nov-2015	11-Nov-2015 – 18-Nov-2015	NA
2016-17	High	1-Mar-2017 – 3-Mar-2017	3-Mar-2017 - 10-Mar-2017	NA
2016-17	Low	22-Feb-2017 – 1-Mar-2017	1-Mar-2017 – 8-Mar-2017	NA
2017-18	High	10-Jan-2018 – 17-Jan-2018	29-Jan-2018 – 5-Feb-2018	NA
2017-18	Low	NA	NA	NA
2018-19	High	22-Mar-2019 – 29-Mar-2019	29-Mar-2019 – 6-Apr-2019	NA
2018-19	Low	NA	NA	NA
2019-20	High	20-Dec-2019 – 27-Dec-2019	13-Jan-2020 – 20-Jan-2020	19-Feb-2020 – 22-Feb-2020
2019-20	Low	20-Dec-2019 – 27-Dec-2019	13-Jan-2020 – 20-Jan-2020	5-Feb-2020 – 8-Feb-2020

Table S2.2: Dataset descriptions and sample sizes used for regression analyses

Dataset	N	Description	Models Used
Full dataset	471	Full dataset, birds with at least 64 visits during the data collection period for sampling behavior, no data from low elevation for 2017-18 or 2018-19	Elevation and year, temperature, snow, age, variation in mean Levins' measures
Sex data	422	Also excludes birds without sex data	Sex
Sex data greater than 8 visits	358	All birds with sex data and greater than 8 visits, including birds from low in 2018-19	Post hoc sex
Spatial cognition data	411	Birds with spatial cognitive data, excludes birds with greater than 3 errors (N = 5)	Spatial cognition analyses
Reversal learning and memory data	316	Birds with spatial cognitive data and reversal learning and memory data	Reversal spatial learning and memory analyses
Repeat birds	184	All birds that were recorded sampling during 2 or more years, 2015-2020	Levins' measures in birds sampling in multiple years
Post-cognitive testing 2019-20	157	Birds that sampled in 2019-20 during the data collection period for sampling behavior before and after cognitive testing	Post-cognitive testing
First year recorded 2016 and 2019	230	Birds that were recorded for the first time in the feeder array system during 2016-17 and 2019-20	Post hoc: age class for first year using feeder arrays
Birds excluded for < 64 visits	94	Birds excluded for visiting less than 64 times (or less than 8 times per day for 8 days). This included N = 94 birds that were excluded from the full dataset entirely.	None

Table S2.3. Number of birds present at each array each year

Array*	Number of Birds Present				
	2015-16	2016-17	2017-18	2018-19	2019-20
High 1	25	45	23	29	74
High 3	43	43	30	50	56
Low 3	37	60	NA	NA	37
Low 8	38	49	NA	14	31

*Array names indicate the elevation (high vs. low) and a position within the field system, not the number of arrays

Table S2.4. Birds at high elevation excluded for visiting fewer than 64 times, by demographics

Age	Sex	High Elevation					Total
		2015-16	2016-17	2017-18	2018-19	2019-20	
Adult	Female	3	4	11	9	2	29
Adult	Male	3	2	22	8	7	42
Adult	Unknown	1	0	5	3	4	13
Juvenile	Male	1	0	10	3	0	14
Juvenile	Unknown	5	1	10	6	3	25
Juvenile	Female	0	2	12	2	0	16
Total		13	9	70	31	16	139

Table S2.5. Birds at low elevation excluded for visiting fewer than 64 times, by demographics

Age	Sex	Low Elevation					Total
		2015-16	2016-17	2017-18	2018-19	2019-20	
Adult	Female	2	0	0	5	3	10
Adult	Male	2	3	0	2	5	12
Adult	Unknown	1	0	0	1	2	4
Juvenile	Male	1	4	0	1	1	7
Juvenile	Unknown	1	5	0	3	0	9
Juvenile	Female	0	6	0	2	0	8
Total		7	18	0	14	11	50

Table S2.6. Linear mixed effects models including number of birds present per array

Fixed effects	Statistics	R ²		
		R ² c	R ² m	Diff.
		0.341	0.206	0.004
Number of birds at array	B = 0.11, F _{1,506.36} = 2.53, P = 0.11			
Total visits	B = 0.33, F _{1,643.85} = 66.95, P > 0.001			
Year	F _{4,543.49} = 13.50, P < 0.001			
Elevation	F _{1,593.54} = 2.86, P = 0.09			
Interaction: year by elevation	F _{2,594.30} = 6.85, P = 0.001			
		0.36	0.227	-0.049
Number of birds at array	B = -0.02, F _{1,603.12} = 0.14, P = 0.71			
Total visits	B = 0.28, F _{1,660.34} = 55.80, P < 0.001			
Average temperature	B = -0.18, F _{1,635.06} = 7.28, P = 0.007			
Mean snow depth	B = 0.21, F _{1,512.54} = 29.67, P < 0.001			
		0.377	0.231	0.004
Number of birds at array	B = 0.09, F _{1,482.11} = 1.55, P = 0.21			
Total visits	B = 0.31, F _{1,638.57} = 55.45, P > 0.001			
Year	F _{4,606.04} = 13.90, P < 0.001			
Elevation	F _{1,607.58} = 0.05, P = 0.83			
Age	F _{1,652.00} = 3.18, P = 0.07			
Interaction: year by elevation	F _{2,607.06} = 3.75, P = 0.024			
Interaction: year by age	F _{4,645.23} = 3.93, P = 0.004			
Interaction: elevation by age	F _{1,642.36} = 0.01, P = 0.92			
Interaction: year by elevation by age	F _{2,650.31} = 2.51, P = 0.08			
		0.358	0.195	0.048
Number of birds at array	B = 0.02, F _{1,356.34} = 0.63, P = 0.43			
Total visits	B = 0.26, F _{1,470.08} = 30.26, P < 0.001			
Year	F _{4,391.19} = 9.56, P < 0.001			
Elevation	F _{1,407.80} = 1.30, P = 0.25			
Age	F _{1,481.54} = 2.49, P = 0.12			
Sex	F _{1,324.00} = 1.08, P = 0.30			
Interaction: year by elevation	F _{2,415.55} = 3.69, P = 0.03			
Interaction: age by sex	F _{1,444.87} = 0.13, P = 0.72			
		0.282	0.209	0.006
Number of birds at array	B = 0.11, F _{1,424.35} = 1.98, P = 0.16			
Total visits	B = 0.29, F _{1,531.21} = 42.73, P < 0.001			
Year	F _{4,477.47} = 9.492, P < 0.001			
Elevation	F _{1,500.36} = 0.24, P = 0.63			
Spatial cognition (scaled)	B = 0.14, F _{1,537.76} = 9.13, P = 0.003			
Interaction: year by elevation	F _{2,490.87} = 5.03, P = 0.007			
		0.355	0.207	-0.002

Number of birds at array	B = -0.04, $F_{1,308.83} = 0.14$, P = 0.71
Total visits	B = 0.29, $F_{1,394.20} = 33.55$ P < 0.001
Year	$F_{4,337.05} = 7.39$, P < 0.001
Elevation	$F_{1,382.05} = 1.46$, P = 0.23
Reversal spatial cognition (scaled)	B = 0.04, $F_{1,395.74} = 0.53$, P = 0.47
Interaction: year by elevation	$F_{2,356.63} = 3.44$, P = 0.03

R²c, R² conditional, R²m, R² marginal, Diff., Difference between R²c and R²m.

Bolded values indicate significance ($\alpha < 0.05$).

*Indicates the difference in R² conditional between the model reported in the table and the equivalent model reported in the main text.

Table S2.7. Linear mixed effects models including number of days each bird participated

Fixed effects	Statistics	R ²		
		R ² c	R ² m	Diff.
		0.346	0.201	-0.004
Number of days birds participated	B = -0.04, $F_{1,658.19} = 1.19$, P = 0.28			
Total visits	B = 0.35, $F_{1,643.42} = 62.14$, P > 0.001			
Year	$F_{4,544.35} = 23.60$, P < 0.001			
Elevation	$F_{1,547.74} = 1.46$, P = 0.23			
Interaction: year by elevation	$F_{1,623.35} = 7.54$, P < 0.001			
		0.381	0.226	0.000
Number of days birds participated	B = -0.02, $F_{1,651.00} = 0.39$, P = 0.53			
Total visits	B = 0.32, $F_{1,639.84} = 59.28$, P > 0.001			
Year	$F_{4,621.19} = 19.25$, P < 0.001			
Elevation	$F_{1,570.51} = 0.07$, P = 0.79			
Age	$F_{1,650.80} = 2.93$, P = 0.09			
Interaction: year by elevation	$F_{2,644.60} = 3.57$, P = 0.029			
Interaction: year by age	$F_{4,644.07} = 3.89$, P = 0.004			
Interaction: elevation by age	$F_{1,640.51} = 0.00$, P = 0.99			
Interaction: year by elevation by age	$F_{2,649.59} = 2.70$, P = 0.07			

R²c, R² conditional, R²m, R² marginal, Diff., Difference between R²c and R²m.

Bolded values indicate significance ($\alpha < 0.05$).

Supplemental Figures

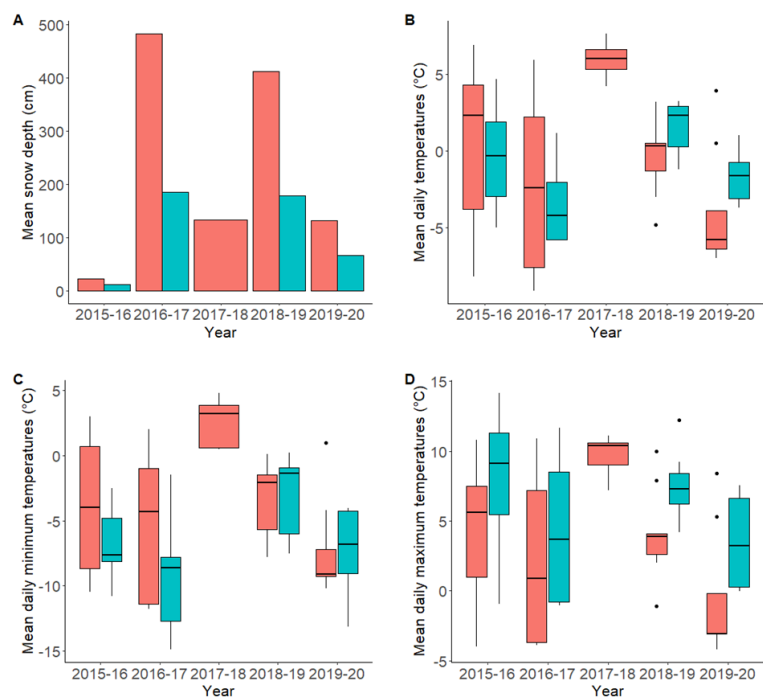


Figure S2.1. Mean conditions during the data collection periods for sampling behavior (2015-2020) at high (pink) and low (blue) elevations. (A) Snow depth, (B) mean daily temperatures, (C) mean daily minimum temperatures, (D) mean daily maximum temperatures. Data from SNOTEL stations 539, 540, 541.

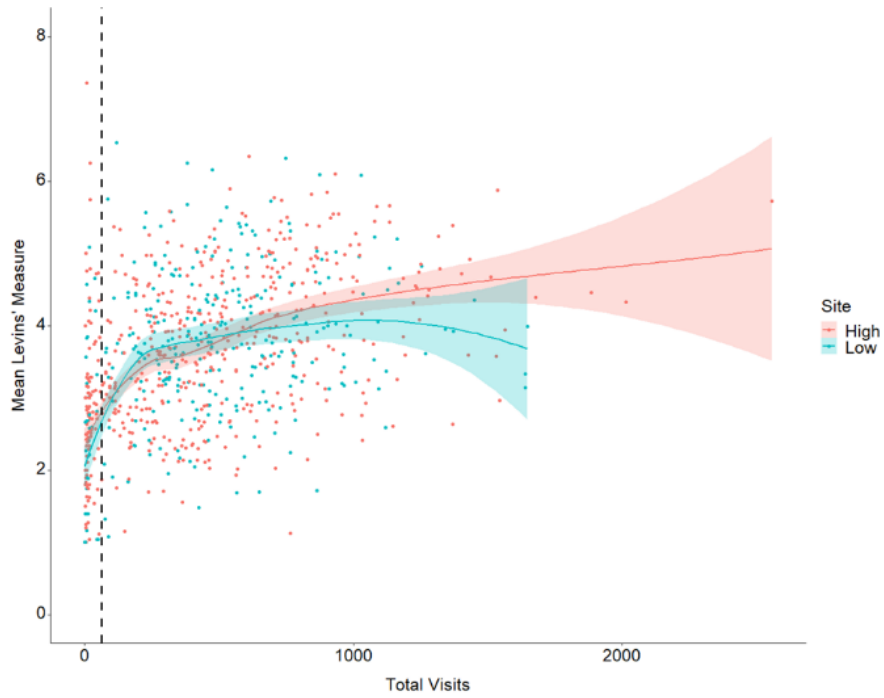


Figure S2.2. Mean Levins' measure by the total number of annual visits by elevation.

Dotted line indicates 64 visits - the criteria for exclusion of some birds due to insufficient number of visits for the analyses. Lines fit with the “loess” smoothing function

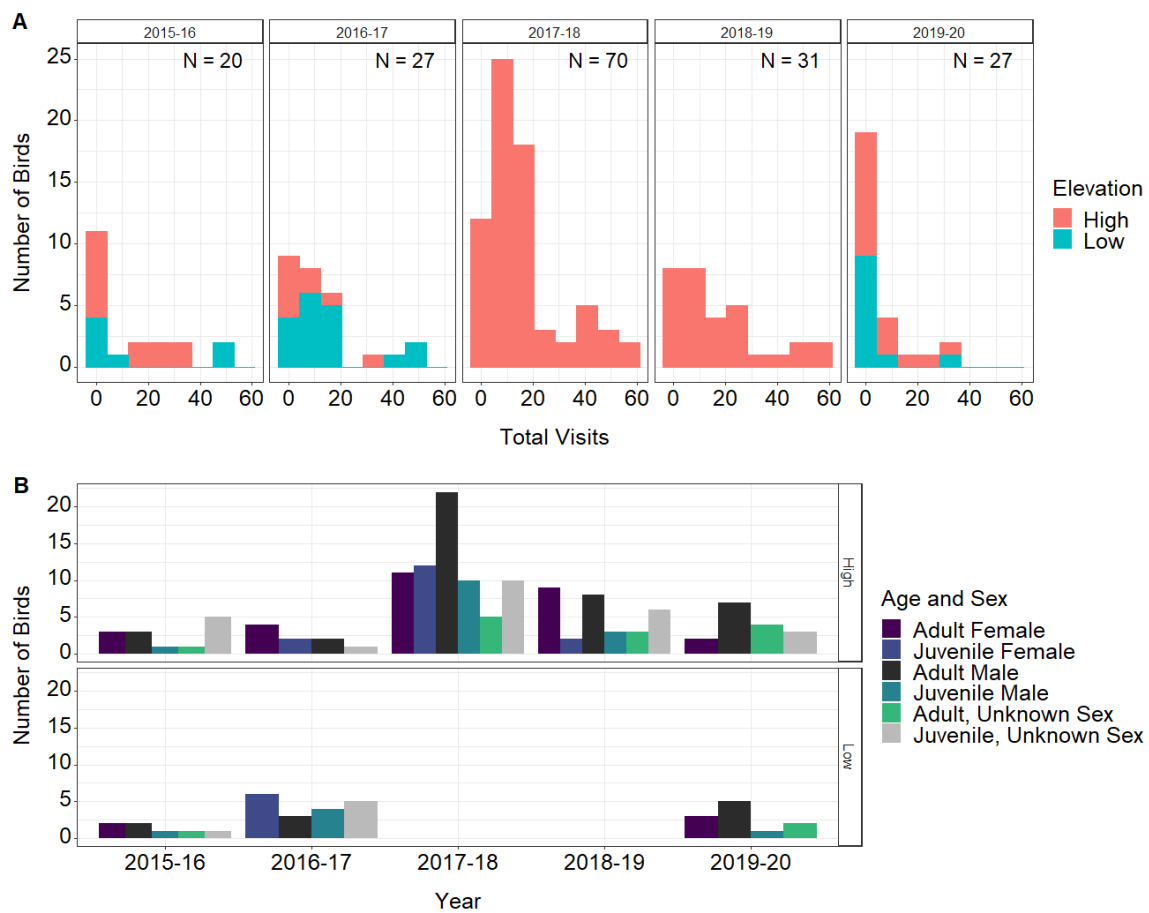


Figure S2.3. Distribution of excluded birds (with < 64 visits) based on the number of visits (A) and the number of birds excluded by their demographics (B).

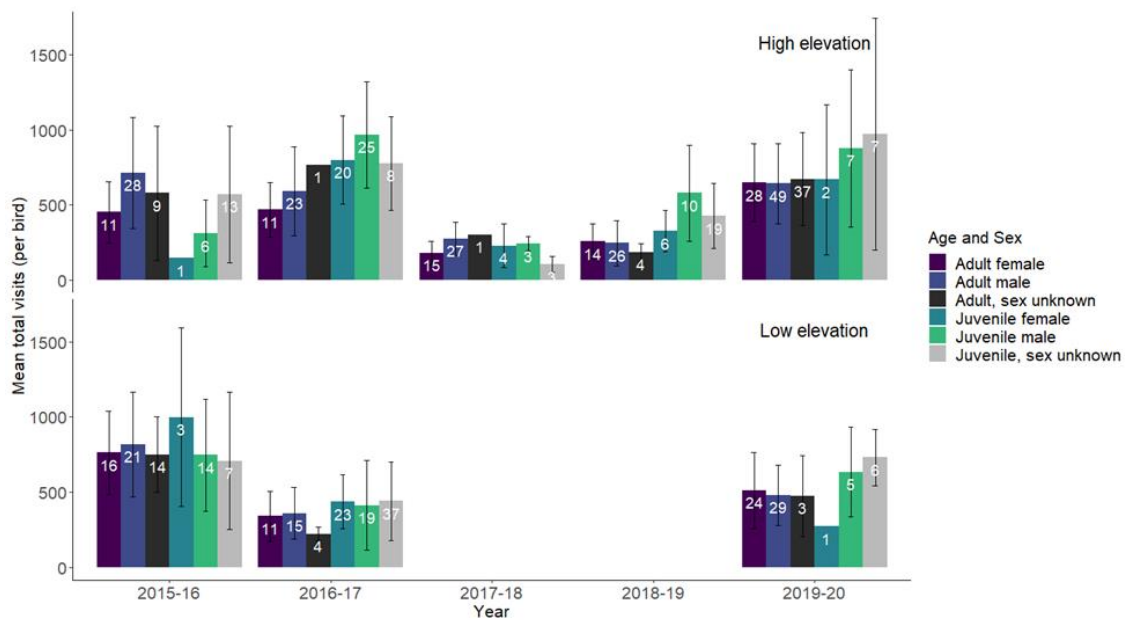


Figure S2.4. Mean number of visits for each data collection period for sampling behavior by age and sex. Numbers indicate the number of birds per group. N = 471.

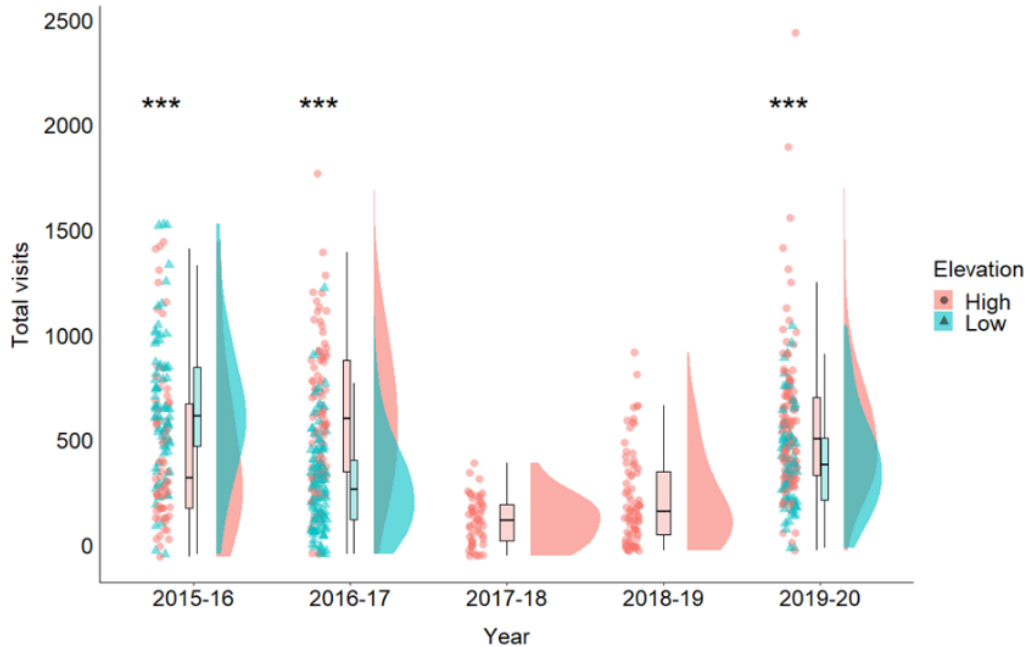


Figure S2.5. Total visits per bird by year and elevation. A linear mixed effects model (R^2 conditional = 0.66, R^2 marginal = 0.29) holding individual bird ID as a random effect showed significant fixed effects of year ($F_{4,354.26} = 66.11$, $P < 0.001$), elevation ($F_{1,486.80} = 17.13$, $P < 0.001$) and an interaction of year by elevation ($F_{2,515.35} = 47.99$, $P < 0.001$). Asterixis indicate significant differences between elevations within the same year (***) indicates significance less than $P < 0.001$). Tukey post hoc pairwise comparisons showed that total number of visits was significantly different at high elevation between 2015-16 and 2016-17 ($P < 0.001$), 2017-18 ($P < 0.001$) and 2018-19 ($P < 0.001$) but not 2019-20 ($P = 0.65$); between 2016-17 and 2017-18 ($P < 0.001$), 2018-19 ($P < 0.001$) and 2019-20 ($P = 0.01$); not between 2017-18 and 2018-19 ($P = 0.47$); and between 2019-20 and 2017-18 ($P < 0.001$) and 2018-19 ($P < 0.001$). At low elevation, 2015-16 was significantly different from 2016-17 ($P < 0.001$) and 2019-20 ($P < 0.001$) but 2016-17 was not significantly different than 2019-20 (0.085). $N = 471$.

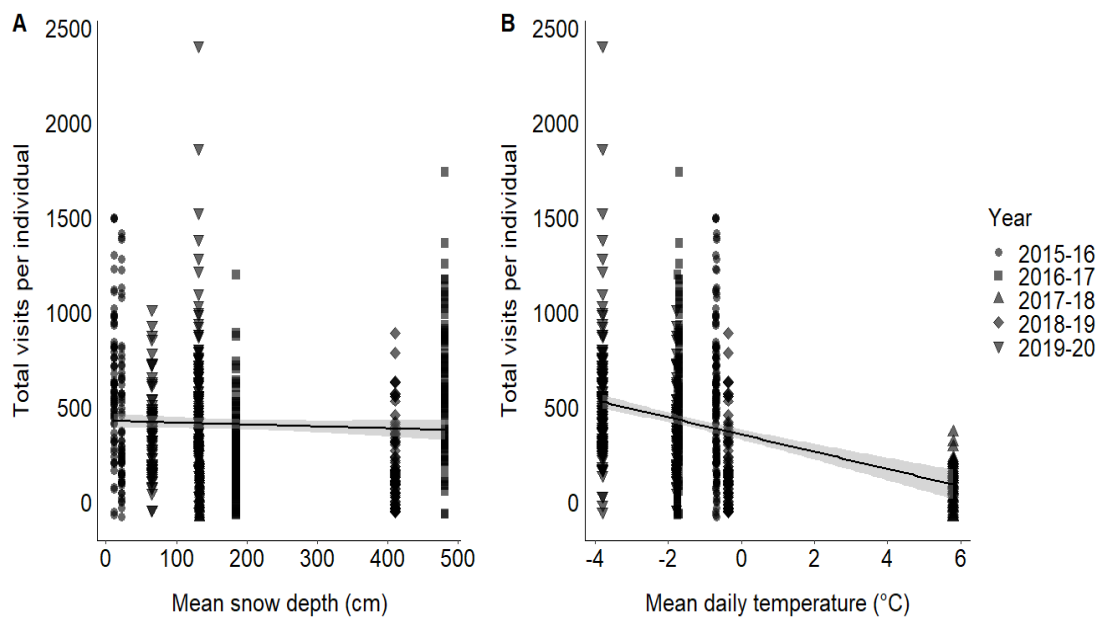


Figure S2.6. Total visits per bird by (A) mean snow depth and (B) mean daily temperature during the period of data collection for sampling. Linear mixed effects model (R^2 conditional = 0.16, R^2 marginal = 0.11) with individual bird ID as a random effect showed that total of visits was significantly less when mean snow depth was higher ($B = -70.91$, $F_{1,526.20} = 25.65$, $P < 0.001$) and when mean daily temperatures were higher ($B = -145.75$, $F_{1,619.05} = 65.21$, $P < 0.001$). $N = 471$.

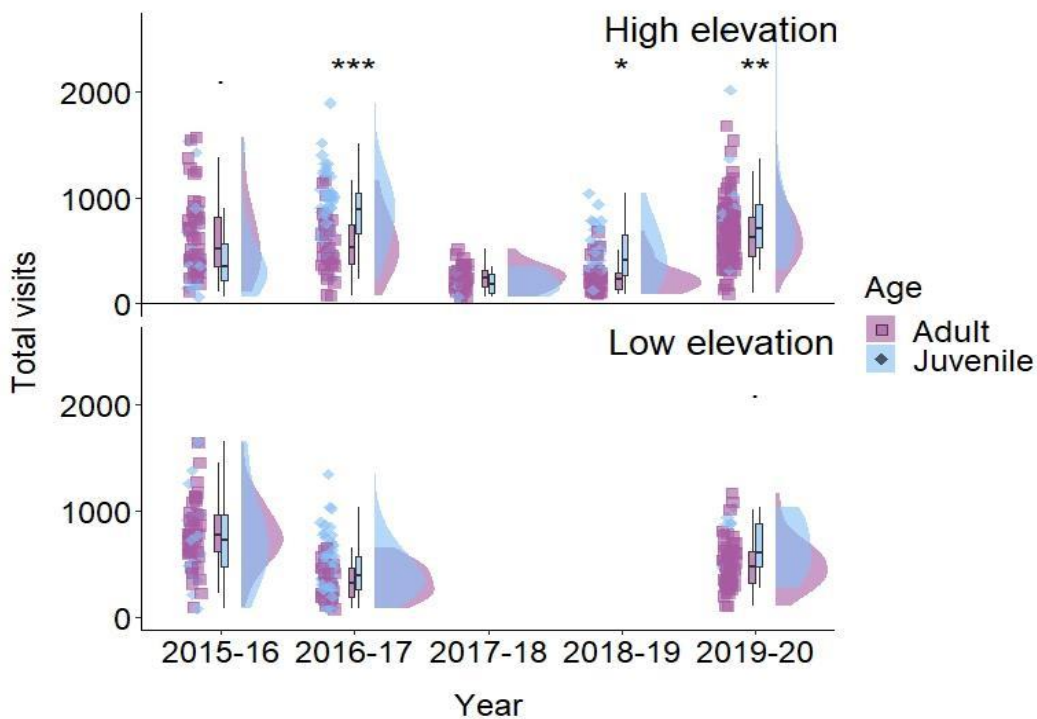


Figure S2.7. Total visits by age, year and elevation. Linear mixed effects model (R^2 conditional = 0.68, R^2 marginal = 0.33) holding individual bird ID as a random effect showed significant fixed effects of year ($F_{4,571.52} = 44.78$, $P < 0.001$), elevation ($F_{1,495.81} = 12.17$, $P < 0.001$), age ($F_{1,646.40} = 10.35$, $P = 0.001$), and fixed interactions of year by elevation ($F_{2,630.67} = 49.32$, $P < 0.001$), year by age ($F_{4,608.06} = 5.61$, $P < 0.001$), and year by elevation by age ($F_{2,644.26} = 4.02$, $P = 0.02$). The interaction of elevation by age was not significant ($F_{1,628.18} = 0.27$, $P = 0.60$). Significance levels from Tukey post hoc pairwise comparisons (within years, between elevations) indicated by stars (‘.’ < 0.06 , ‘*’ $< .05$, ‘**’ $< .01$, ‘***’ $< .001$). $N = 471$.

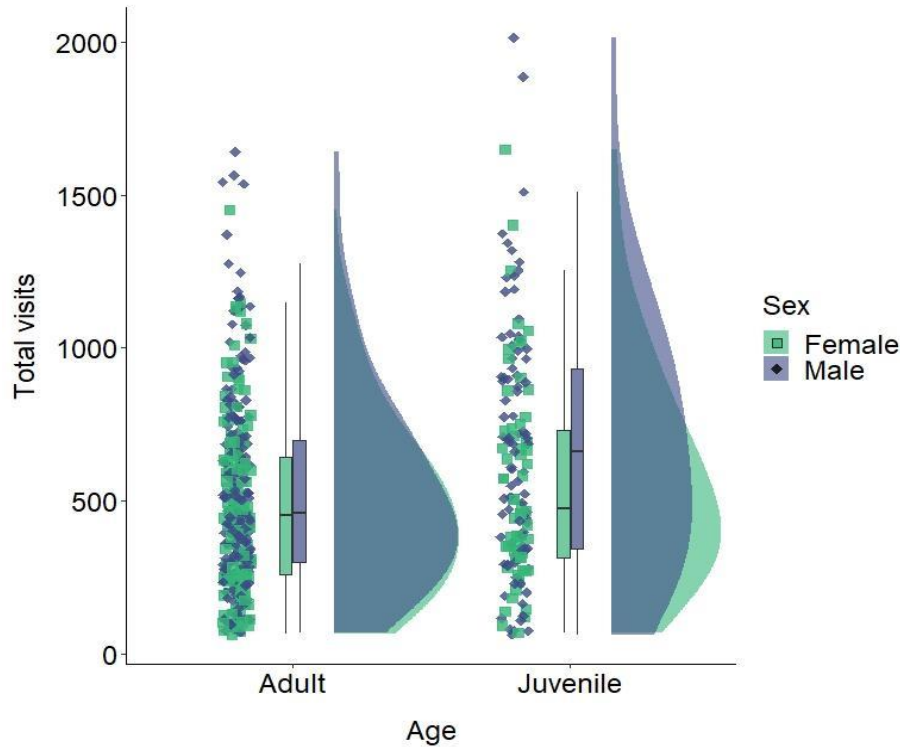


Figure S2.8. Total visits per individual by age and sex. A linear mixed effects model (R^2 conditional = 0.67, R^2 marginal = 0.35) with individual bird as a random effect showed no significant difference of sex ($F_{1,307.71} = 3.07$, $P = 0.08$) or age by sex interaction ($F_{1,372.98} = 0.08$, $P = 0.78$). There were significant effects of age ($F_{1,456.98} = 17.80$, $P < 0.001$), year ($F_{4,291.33} = 57.51$, $P < 0.001$), elevation ($F_{1,329.79} = 14.52$, $P < 0.001$) and the interaction of year by elevation ($F_{2,383.03} = 37.92$, $P < 0.001$) were significant. $N = 321$.

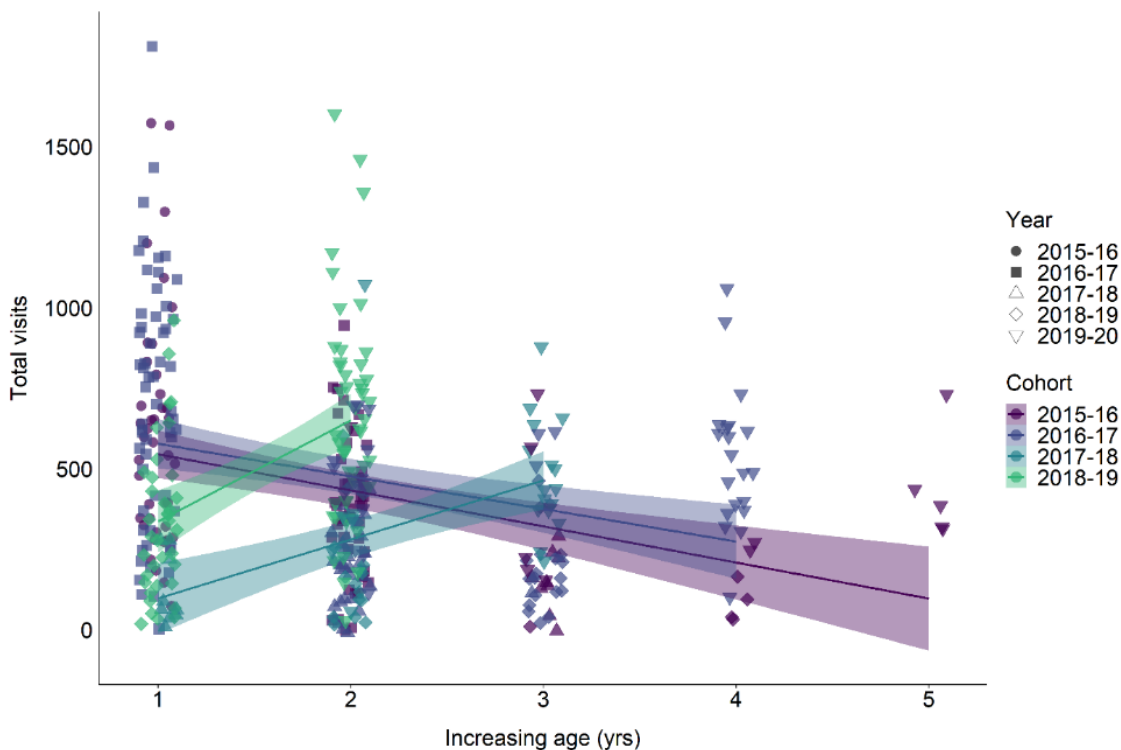


Figure S2.9. Total visits by increasing age in years. A linear effects model (R^2 conditional = 0.54, R^2 marginal = 0.36) with individual bird as a random effect showed no significant effect of increasing number of years of experience ($F_{1,371.68} = 0.27$, $P = 0.6$) and a significant effect of cohort ($F_{3,226.59} = 6.29$, $P < 0.001$) and year ($F_{4,262.60} = 56.99$, $P < 0.001$).

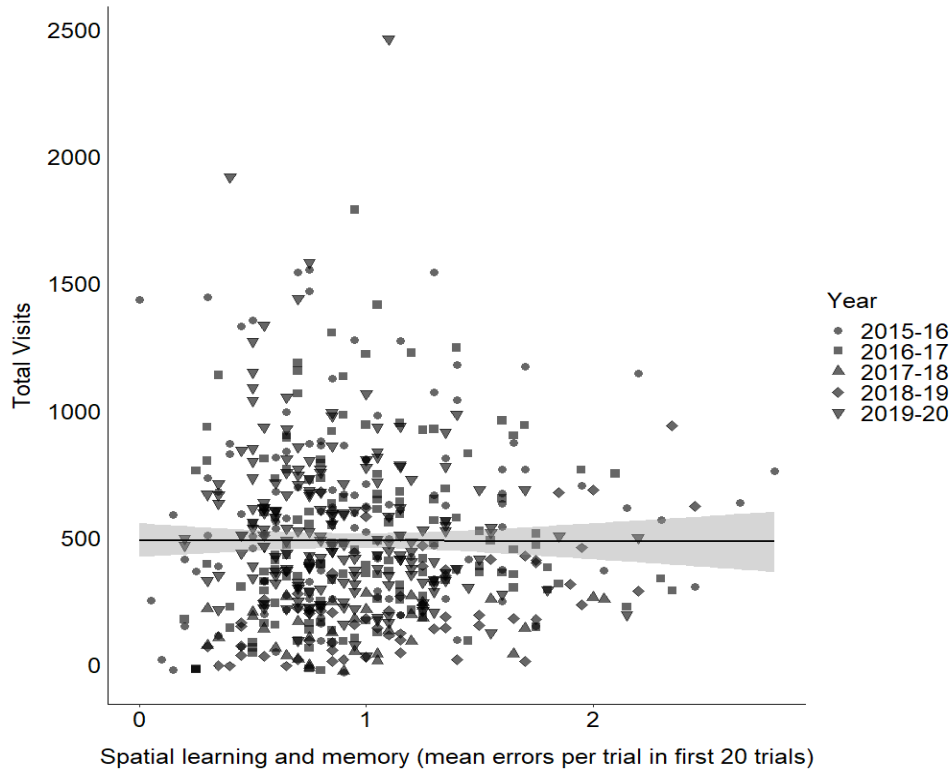


Figure S2.10. Total visits per individual and spatial learning and memory ability. A linear mixed effects model (R^2 conditional = 0.59, R^2 marginal = 0.28) with individual bird as a random effect showed significant differences in year ($F_{4,310.61} = 48.42$, $P < 0.001$), elevation ($F_{1,426.70} = 16.34$, $P < 0.001$), and interaction of year by elevation ($F_{2,454.38} = 27.31$, $P < 0.001$), but no significant difference in spatial learning and memory ability ($\beta = 27.39$, $F_{1,518.78} = 3.40$, $P = 0.07$). $N = 411$.

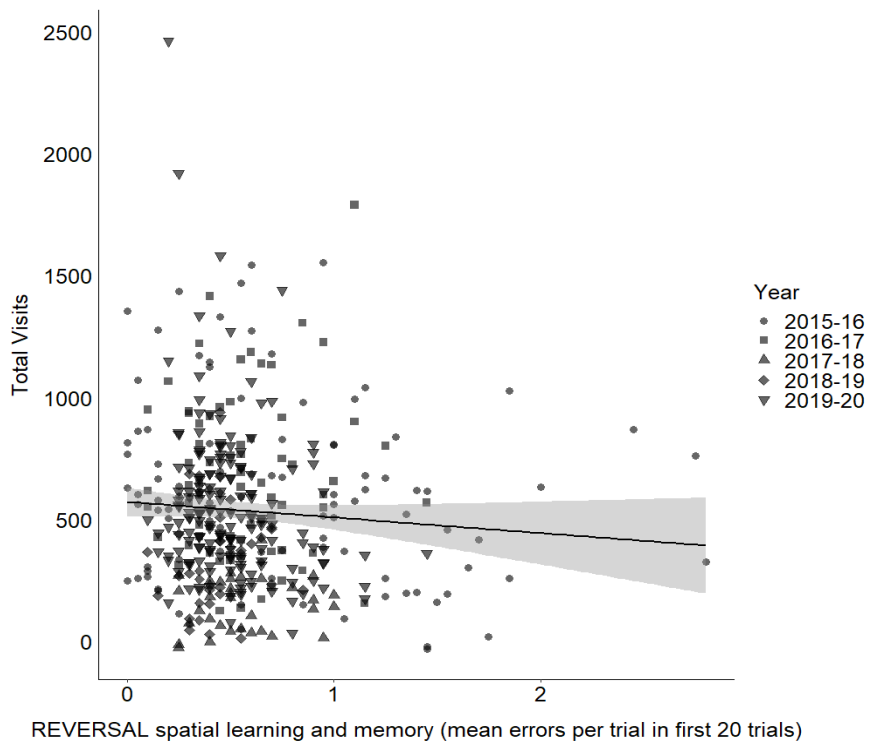


Figure S2.11. Total visits per individual by reversal spatial learning and memory ability.

A linear mixed effects model (R^2 conditional = 0.58, R^2 marginal = 0.26) with individual bird as a random effect showed that birds visited less also made more mean errors per trial in the first 20 trials ($\beta = -40.08$, $F_{1,386.73} = 3.92$, $P = 0.05$). There were still the significant effects of year ($F_{4,209.87} = 31.36$, $P < 0.001$), elevation ($F_{1,375.18} = 9.82$, $P = 0.002$), and interaction of year by elevation ($F_{2,316.45} = 15.82$, $P < 0.001$). $N = 316$.

Chapter 3 Food-Caching Chickadees Do Not Exhibit Directional Bias When Learning a Spatial Task

L.M. Benedict^{1*}, V.K. Heinen¹, B.R. Sonnenberg¹, A.M. Pitera¹, E.S. Bridge², V.V. Pravosudov¹

¹Department of Biology and Ecology, Evolution and Conservation Biology Graduate Program, University of Nevada, Reno, Reno, NV, U.S.A.; ²University of Oklahoma, Oklahoma Biological Survey, Norman, OK, U.S.A.

*Corresponding author, Lauren Benedict Nguyen, published as L.M. Benedict

Publication Citation

Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., & Pravosudov, V. V. (2023). Food-caching chickadees do not exhibit directional bias when learning a spatial task. *Behavioral Ecology and Sociobiology*, 77:4. doi: 10.1007/s00265-022-03275-6

Abstract

Animals frequently encounter situations in which they can choose to move either left or right. Consistent preferences to move a specific direction may be associated with lateralization, or the asymmetric structure and function of the brain and nervous system. Other lateralized behaviors commonly occur across taxa, possibly reflecting a selective advantage of cerebral specialization. Yet lateralization and possible directional biases are rarely tested within an ecologically relevant context, such as movement, or while animals are making decisions on a larger scale. Here, we quantify to what extent wild food-caching mountain chickadees (*Poecile gambeli*) in their natural environment demonstrate consistent directional biases in movement when learning a spatial task. Directional bias was estimated from the direction (left or right) that birds moved around a square experimental apparatus while searching for a food reward at the beginning of the tasks, at which point birds had not yet fully learned the location of the food reward. Chickadees did not show directional bias in movement at a population level. Individual variation in directional bias was significantly repeatable across years but did not significantly vary between two elevations and was not significantly associated with performance on either a spatial learning and memory task or a single spatial reversal learning task. Overall, our results show that chickadees did not show directional bias when deciding what direction to move during spatial cognitive tasks, suggesting that no consistent preference in movement direction may be advantageous when searching for food on a larger scale.

Significance Statement

Many animals across a wide range of taxa will consistently prefer to use either their left or right side to complete certain types of tasks. Such asymmetric behaviors may be associated with asymmetries in brain structure and are well documented in birds. Yet mountain chickadees did not show similar directional biases in their movement-based decision-making. Furthermore, biases in their movement were not associated with overall cognitive performance. These null results suggest that while strong left or right preferences may be beneficial in certain contexts, such biases might not be advantageous while foraging for food on a larger scale.

Introduction

Animals regularly experience situations in which they must make decisions about where to go and what direction to move within their environments. Such decisions often involve choosing to move left or right from their starting position, such as when departing a perch, moving around an obstacle, or searching for food. However, it is unclear whether animals may have directional preferences that could bias these movement-related decisions. In other behaviors, there is widespread evidence across taxa that animals have directional preferences that are consistent for certain tasks or contexts (reviewed in Rogers et al., 2013); for example, some nonhuman primate species preferentially use their right hand while solving two-handed tasks (Hopkins et al., 2011), poeciliid fish consistently turn to the left to avoid a predator and to the right to detour around a barrier (Bisazza et al., 1998b) and wild pigeons preferentially use one field of vision at a time to forage on different food types (Karenina and Giljov, 2022). But little is known about how these directional biases might scale up to affect movement-related decisions. Individual biases in movement may be important to understand, particularly because many behavioral studies in both laboratory and field conditions record behaviors that are movement-based. For example, many spatial tasks require an individual to move around a circle (e.g., Croston et al., 2016; Gawel et al., 2019). If animals exhibited a significant preference to move left or right, such preferences might introduce a bias to estimates of learning performance involving circular search.

Many directional biases have been associated with lateralization, or the asymmetric structure and function of the brain and nervous system (Rogers et al., 2013).

Processes that are specialized to one side of the brain may result in control of that hemisphere over related behaviors (Rogers, 2021). For example, in many vertebrates, information collected by each eye is processed almost entirely by the opposite hemisphere (Rogers, 2021). Thus, lateralization has been shown in visual-based behaviors that involve preferentially using one eye or field of vision to collect different types of information (Clayton and Krebs, 1993, 1994; Jozet-Alves et al., 2012; Tommasi et al., 2000; Loconsole et al., 2021) or to collect information in different contexts (Robins and Rogers, 2004; Ventolini et al., 2005; Zucca and Sovrano, 2008; Shen et al., 2019). Directional biases in motor-based behaviors may also suggest lateralization, such as using one limb to hold food or manipulate objects (Bisazza et al., 1996; Magat and Brown, 2009; Brown and Magat, 2011; Hopkins et al., 2011; Zhao et al., 2012; Bell and Niven, 2016; Isparta et al., 2020; Leaver et al., 2020) or consistently turning in one direction (Bisazza et al., 2000, 2001; Miler et al., 2017). However, although general trends have begun to develop (Rogers, 2021), the literature is mixed about to what extent lateralized behaviors become fixed across populations (e.g., Hopkins, 2006). More typically, individuals vary in both the direction (i.e., left or right) and strength (i.e., frequency of behavior in the same direction) of the lateralization.

Many behavioral studies show that individuals with strongly lateralized behaviors perform better on cognitive tasks than more weakly lateralized individuals, including in associative learning (honeybees in Letzkus et al., 2008; larval antlions in Miler et al., 2017), visual discrimination (parrots in Magat and Brown, 2009; wild American robins in Scharf et al., 2019; wild pigeons in Karenina and Giljov, 2022), numeric counting (guppies in Dadda et al., 2015), problem solving (cats in Isparta et al., 2020) and dual

tasks, such as predator vigilance while completing a foraging visual discrimination task (chicks in Rogers et al., 2004; marmosets in Piddington and Rogers, 2013). However, other studies find negative (topminnows in Dadda et al., 2009; wild squirrels in Leaver et al., 2020) or null (pheasants in Whiteside et al., 2020) relationships between lateralized behaviors and cognitive abilities. These contrasting results suggest that there may be costs to lateralization (pheasants in Whiteside et al., 2018), or that non-cognitive factors might influence individual variation in directional biases (reviewed in Güntürkün et al., 2020; Rogers, 2021). For instance, lateralized behaviors imply lateralized practice and learning with both perceptual and motor-based systems (reviewed in Güntürkün et al., 2020), potentially leading to improved motor control (locusts in Bell and Niven, 2016) or decreased reaction times (fish in Dadda et al., 2010). Moreover, some lateralized behaviors appear task-dependent (parrots in Schiffner and Srinivasan, 2013; Trinidadian guppies in Penry-Williams et al., 2022) and highly plastic, as individuals can adjust the strength of laterality based on the cognitive demands of the task (wild pigeons in Karenina and Giljov, 2022) or predation risk (damsel fish in Ferrari et al., 2017). Ultimately, it is important to understand how these directional biases might affect decision-making in animals, which has gotten less attention in the literature.

Here, we used data from three types of spatial cognitive tasks performed by wild food-caching mountain chickadees (*Poecile gambeli*) in their natural environment to assess whether chickadees show directional biases in movement while learning these spatial tasks. We also wanted to explore how possible directional biases might (a) be associated with differences in individual spatial cognitive performance, (b) vary with environmental conditions across two montane elevations and (c) be repeatable across two

consecutive years. All three cognitive tasks used the same 8-feeder array setup, with feeders positioned equidistantly on a square frame (Fig. 3.1). We estimated directional bias as birds searched for a single rewarding feeder at each array, using the order of feeders visited to estimate each bird's movement around the feeder array. Movement around a barrier has commonly been used to assess lateralization in fish through detour tasks (Bisazza et al., 1998a; Penry-Williams et al., 2022). Our method to estimate directional bias was similar: after visiting any unrewarding feeder at the array, birds could choose to move to equidistantly positioned feeders on the left or right to search another feeder for food (Fig. 3.1). Birds could clearly view both adjacent feeders from any given feeder perch. In this system, chickadees arrive at any feeder in the array and then move around the array to sample different feeders until they discover the single rewarding feeder. Birds do not leave the array until they have obtained food at the rewarding feeder. Although the literature suggests that while lateralization and other directional biases could affect the learning process (reviewed in Rogers, 2021), there is no evidence that the learning process could affect movement bias. However, we used a conservative approach and only analyzed data when the birds had not yet fully learned the location of the correct rewarding feeder, and so had to search the feeder array to find the food reward. If any movement bias exists, it should be evident in continuous movement among feeders and so we used the data with birds visiting at least 2 unrewarding feeders before finding a food reward, as this allowed us to examine direction of 2+ moves (see Table 3.1 for terminology).

Cognition-Based Predictions

Chickadees are food-caching birds that rely on spatial learning and memory to hide and recover food caches during winter. We would expect to find directional biases in behaviors related to spatial cognition in chickadees because the region of the brain related to spatial cognition in avian species (hippocampal formation) has specialized functional structures (Tommasi et al., 2003; Siegel et al., 2006) and spatial information is processed differently when collected from the left and right visual fields (Clayton and Krebs, 1993; Tommasi et al., 2003). Furthermore, in food-caching species there appears to be an asymmetrical transfer of spatial information from one side of the brain to the other (Clayton and Krebs, 1993). If mountain chickadees have a directional bias while learning in a cognitive task, we would expect that over 50% of birds would show a movement bias in the same direction and that this bias would be repeatable within individuals between years. Furthermore, if these directional biases are associated with spatial cognitive ability, then we would expect that performance on spatial cognitive tasks should be associated with the strength of individual biases.

Environment-Based Predictions

We conducted this study in a montane system in which we have previously found significant cognitive, morphological and behavioral differences between mountain chickadees from high (~2400m) and low (~1900m) elevations, as well as significant

differences in winter environmental harshness and predictability across elevations (Branch and Pravosudov, 2016; Croston et al., 2016, 2017; Kozlovsky et al., 2018; Pitera et al., 2018; Tello-Ramos et al., 2018; Sonnenberg et al., 2019; Benedict et al., 2021). Compared to birds at milder, lower elevations, birds at harsher, higher elevations experience a longer duration of snow cover (Kozlovsky et al., 2018) and less predictable foraging conditions, likely due to interruptions from frequent and variable winter storms (Pitera et al., 2018). Birds at high elevations also usually perform better on spatial learning and memory tasks (Croston et al., 2016) and appear less cognitively flexible (Tello-Ramos et al., 2018). If directional bias varies with environmental harshness, or with different demands of the environments at these two elevations, we expect to see a difference in the strength and possibly the direction of movement between birds from each elevation.

Sex-Related Predictions

During the cognitive tasks, birds participated willingly and there was no strict control for how many birds could attempt to forage from the arrays at the same time. This raises the possibility that more dominant birds could have potentially displaced less dominant birds trying to visit certain feeders. Social dominance in chickadees and other Paridae species follows a linear hierarchy in which adult and juvenile males are socially dominant over females (e.g., Ekman, 1989). Thus, if the direction that birds move around the arrays was affected by social dynamics rather than passive directional biases, then we would expect females to show weaker directional bias than males due to more frequent

displacement by dominant birds. However, the study design limited the likelihood of displacement events: (1) birds were assigned to different rewarding feeders to distribute visits across all 8 feeders; (2) the study only analyzed instances when birds moved away from unrewarding feeders after making a location error, meaning that dominant birds would not gain a food reward by displacing birds from these unrewarding feeders and should not be motivated to do so; and (3) we estimated directional bias both as the initial direction that birds moved after first arriving at the feeder arrays and as the consistency in direction, if birds moved twice in the same direction. The consistent directional bias should be more robust to displacement by other birds than an initial bias measured from one movement alone; It is unlikely that a bird would keep moving in the same direction following a displacement and that a bird would be consistently displaced at feeders that do not provide food. Finally, our previous work showed that social dominance status did not affect performance on these spatial cognitive tasks, measured as the number of location errors before visiting the rewarding feeder (Heinen et al., 2021)

Methods

Study System

All data were collected as part of a long-term study (2014 - ongoing in 2022) of mountain chickadees at Sagehen Experimental Forest in the Sierra Nevada mountains (Sagehen Creek Field Station, University of California Berkeley), located 10 km north of Truckee, CA, USA. (Freas et al., 2012; Croston et al., 2016, 2017; Kozlovsky et al.,

2018; Tello-Ramos et al., 2018). Within this system, there are substantial differences in winter conditions between higher (ca. 2400m) and lower (ca.1900m) elevations: conditions at higher elevation are consistently harsher, characterized by lower ambient temperatures, longer duration of snow cover, more unpredictable interruptions in food availability due to more frequent and unpredictable snowfall and more severe storms (Kozlovsky et al., 2018; Pitera et al., 2018). Annual banding efforts, nestbox breeding surveys, and cognition experiments were concentrated at the high and low elevation sites to explore elevational differences since 2013. Birds were trapped at nestboxes during summer or at established bird feeders using mistnets in the fall and winter. Trapped birds were banded with color bands including a colored passive integrated transponder (PIT)-tag with a unique alphanumeric ID (IB Technology, Leicestershire, U.K.). In this study system, sex (male or female) was determined from previous summer breeding survey records using physiological and behavioral evidence (e.g., brood patch or cloacal protuberance, song), if possible (Meigs et al., 1983; Pyle, 1997).

Experimental Apparatus

The data used in this study were collected in winter 2019-20 and winter 2020-21 as part of a long-term effort to test spatial cognitive performance in mountain chickadees. The data were collected using four spatial feeder arrays (two arrays per elevation, ca. 1.2 km apart) established in 2014 (Croston et al., 2016, 2017). Each array consisted of eight “smart” feeders mounted equidistantly to a square 1.2m x1.2m aluminum frame raised ca. 3m above the ground. Feeders were equipped with a radio frequency identification

(RFID) data logger connected to a perch-mounted antenna that detected and logged the passive integrated transponders (i.e., PIT tags) of birds that landed at the feeders. Each feeder had a mechanized door to control access to a supply of black oil sunflower seeds (Croston et al., 2017; Tello-Ramos et al., 2018; Bridge et al., 2019). Feeders could be set to three different modes: (1) “open” mode, in which feeder doors were always open; (2) “all” mode, in which feeder doors were closed until any PIT-tagged bird triggered the door to open by landing on the feeder perch; and (3) “target” mode, which was similar to “all” mode except that each bird could only access food from one of the eight feeders, though all feeders recorded the time and PIT tag for all visits. “Open” and “all” mode were used to habituate birds to the feeders before annual cognitive tasks, whereas “target” mode was used during cognitive tasks to assign birds to one rewarding feeder each. Birds could forage during daylight hours, but feeders automatically turned off after sunset and turned back on before dawn, ca. 20:00 to 06:00).

These 8-feeder arrays had not been used before to test directional biases or laterality but are well suited to measure such biases in movement. The feeder arrays allow animals freedom of movement while still forcing animals to make a left or right decision to move between feeders. When a chickadee arrives at any of the array feeders, it continues moving around the array to sample the feeders until it lands on the single rewarding feeder. Birds usually do not leave the array until they find a rewarding feeder. When a bird lands on any feeder, it faces the feeder door which may open to provide access to food. If the door does not open (as in Fig. 3.1B) the bird usually moves left or right to the next closest feeder. In contrast, many other laboratory studies restrain animals to some degree, so animals can only use one eye or one leg for a given behavior. While

those study designs collect invaluable data, our study design allows us to observe directional biases at a different scale, by observing the final decision of where the animal chooses to move given all options. Furthermore, the “smart” feeders are an advantage of our study design, as these feeders automatically recorded visits of PIT-tagged birds and controlled individual access to the feeders. As such, our data were collected blind in respect to individual performance and cognitive metrics. It was not possible to record data blind with respect to elevation because our study was conducted in the field with different arrays at different elevations. Additionally, birds could participate in any number of trials during our study, each trial starting when a bird approached the array (i.e., visited any feeder) and ending when the bird visited the correct rewarding feeder and received a food reward (3.1). Birds were likely motivated to participate in multiple trials because chickadees typically forage for seeds one-at-a-time, leaving the array to consume or cache the seed after each successful visit (Croston et al., 2017; Tello-Ramos et al., 2018). As a food-caching species, chickadees cache rather than consume the majority of seeds obtained during trials; thus, motivation likely did not diminish during successive trials (e.g., Croston et al., 2016).

Estimating directional bias

Directional bias was estimated from the direction (left or right) that PIT-tagged birds moved around the feeder arrays, inferred from subsequent visits recorded automatically at smart-feeders. Visits were not validated by visual observations to ensure that they represented real movements between feeders. But chickadees do not leave the

array until they get a food reward from the correct feeder, and so typically sample multiple unrewarding feeders in a row only leaving the array after they obtain food. Supporting these observations, the mean trial time for trials in which birds visited at least 2 unrewarding feeders was only 39.5 ± 73.9 seconds. Given the small amount of time it takes to complete a trial, it is unlikely that chickadees could frequently leave the feeder array to perch nearby (arrays were suspended in the air ca. 3m high and were >3m away from nearby trees on all other sides). This suggests that visit data indicated movement at the beginning of each trial. Each trial was scored for two estimates of directional bias: (1) initial bias, or the direction the bird initially moved after starting a trial at an incorrect, unrewarding feeder, and (2) consistent bias, the direction the bird consistently moved after its initial movement, if it moved in the same direction again after visiting the first unrewarding feeder and then after visiting the second unrewarding feeder (Fig. 3.2). Both types of directional bias were measured as “left” or “right” from the viewpoint of a bird at the feeder (Fig. 3.1, 3.2).

A primary goal of the study was to describe directionality of movement during foraging-based search as birds were learning the location of the rewarding feeder in each task. To do this, we had to only use trials in which birds visited at least 2 unrewarding feeders before visiting the rewarding feeder (i.e., made at least 2 location errors) during “target” mode. The 2020-2021 sample size was reduced from 543,878 trials completed by 321 birds across all cognitive tasks to 16,712 trials completed by 316 birds across all tasks (Table A3.1). While this might seem like a dramatic reduction, 89.5% of the excluded trials showed near perfect performance, in which we could not score direction because birds only visited the correct rewarding feeder before leaving the array. Using a

minimum threshold of 2 or more location errors per trial was necessary because: (1) these birds made at least 3 visits to feeders per trial and thus could be scored for consistent bias, which could only be estimated from at least 3 visits; (2) initial bias was always estimated when birds moved between 2 unrewarded locations (e.g., errors), avoiding any movement directly to the correct rewarding feeder from the adjacent feeder, which might reflect other processes; and (3) the exclusion ensured that birds were all at relatively the same point in the learning process when the data were collected, by eliminating the trials in which birds were performing perfectly or nearly perfectly (making 1 or no location errors). Making 2 or more location errors indicates that the birds had not yet fully learned the rewarding location, considering that, on average, chickadees typically make 1 or fewer errors after the first few trials (Croston et al., 2018; Sonnenberg et al., 2019; Tello-Ramos et al., 2019). Moreover, trials with only 1 location error are more ambiguous to analyze because while the single location error could be due to imperfect memory, it could also be due to birds waiting for the target feeder to be available. As our data were collected automatically, we do not know how frequently this might have occurred; but using a minimum of two location errors and including consistent bias estimates should reduce this in the dataset. It is important to note that the 2+ location error cutoff did not remove birds from the dataset based on learning ability, because all birds make errors at the beginning of the cognitive tasks while they learn. In addition, there is no evidence that the learning process can affect directional bias or laterality, whereas previous studies have suggested that directional bias could be expected to affect learning (Güntürkün et al., 2020, Rogers, 2021).

To address our main study question, we needed to use all trials to analyze whether directional bias was apparent at the decision-making level, so that we could analyze behavior while birds were learning each task. Using all trials also provided the largest sample size and the most statistical power to detect any potential movement bias. But to explore whether directional bias might be present before birds even started learning during each task, we also estimated initial and consistent bias using only the first trial of each task (i.e., after a new feeder location was assigned). During these “first trials,” birds had no knowledge of the correct feeder location and were about to begin the learning acquisition stage. In the serial reversal tasks, we included trials after every switch of the rewarding feeder. This subset comprised 26.7% of the all-trial initial bias dataset (N = 269) and 25.5% of the all-trial consistent bias dataset.

Additionally, we excluded any trials in which a bird crossed to the opposite side of the array during the first three visits per trial. This was largely because we could not determine direction if the bird moved to the feeder directly across from it, and partially because moving across the entire array likely introduces more opportunity to be displaced by other birds and could be driven by other factors. To be conservative, we only used trials in which birds visited feeders that were no farther apart than two feeders. In 2020-2021, this excluded one bird and overall removed 2055 trials across all cognitive tasks from the initial dataset and excluded three birds and overall removed 5783 trials from the consistent bias dataset across all cognitive tasks (Table A3.1).

Quantifying Directional Bias

Directional bias (both initial and consistent) was quantified using a laterality index (LI, Eq. 3.1), using the total number of left-scored (L) and right-scored trials (R) per bird:

$$LI = \frac{L-R}{L+R} * 100 \quad (\text{Eq. 3.1})$$

The sign of the bias index (LI) represents the direction of the bias (negative numbers indicate “right,” positive numbers indicate “left”) and the value represents the strength (LI = ± 100 indicates that birds almost always moved the same direction, and LI = 0 indicates that birds went left and right relatively equally). To estimate the strength of laterality regardless of direction, the absolute value of the bias index was used, ranging from weak to strong [0, 100] (Penry-Williams et al., 2022).

This index did not account for the total number of trials analyzed per bird, which ranged from 7 to 120 trials per bird across all cognitive tasks in the 2020-2021 initial bias dataset (mean = 48 ± 21 SD trials per bird) and from 6 to 65 trials per bird across all cognitive tasks in the 2020-2021 consistent bias dataset (mean = 27 ± 13 SD trials per bird). For 2019-2020, the datasets ranged from 6 to 221 trials per bird across all cognitive tasks for the initial bias dataset (mean = 34 ± 32 SD trials per bird) and from 6 to 44 trials per bird across all cognitive tasks in the consistent bias dataset (mean = 15 ± 7 trials per bird).

Directional Data

To assess possible directional bias, we used data collected from spatial cognitive tasks during the winter season of 2020-21: a spatial learning and memory task (January 13-17, 2021), a single reversal learning task (January 17-20, 2021) and two serial reversal learning tasks (January 20-26, 2021; February 10-26, 2021). Additionally, to estimate the repeatability of these bias scores, we used data collected from spatial cognitive tasks during the winter season of 2019-20: a spatial learning and memory task (high elevation: February 2-7, 2020; low elevation: January 20-24, 2020) and several single reversal learning tasks (high elevation: February 7-13, 2020 and February 24-28, 2020; low elevation: January 24-29, 2020 and February 10-14, 2020). These 2019-20 data were only used for repeatability of directional biases within individuals between two consecutive years.

Spatial Learning and Memory Task and Single Reversal Learning Task

Cognitive performance scores were used from two of the cognitive tasks conducted in 2020-2021 to explore how individual laterality scores might affect cognitive performance: spatial learning and memory ability and single spatial reversal learning ability. These two cognitive tasks were conducted consecutively following previously established protocols using the 8-feeder arrays (Croston et al., 2016, 2017; Tello-Ramos et al., 2018; Sonnenberg et al., 2019). Performance from the serial reversal tasks was not

used because this performance was assessed through additional metrics that were not directly comparable to the other two cognitive tasks.

For the spatial learning and memory task (January 13 - 17, 2021), feeders were switched from “all” mode to “target” mode, restricting PIT-tagged birds from accessing all 8 feeders to just one. PIT-tagged birds were pseudorandomly assigned to rewarding feeders so that no bird was assigned to its most frequently visited feeder from “open” or “all” mode. The number of birds assigned to each feeder was relatively equal. Immediately following the spatial learning and memory task, the single spatial reversal learning task began by switching the rewarding feeder assignments for each bird to a new feeder on a different side of the square feeder array (January 17-20, 2021). To minimize social learning, birds that were assigned together to the same rewarding feeder in the spatial learning and memory task were individually assigned to separate rewarding feeders during the reversal task.

For both cognitive tasks, better performance was indicated by a lower number of mean location errors per trial in the first 20 trials. A trial started when a bird visited any feeder in the array and ended when the bird landed on the rewarding feeder and obtained food (Croston et al., 2017). Location errors were measured by the number of unrewarding feeders visited within a trial prior to visiting the rewarding feeder (Croston et al., 2017). Our previous work indicated that the mean performance across the first 20 trials provided a good point of comparison between individuals’ cognitive performance, as it is directly related to survival (Sonnenberg et al., 2019).

Data Exclusions

For statistical purposes, we only analyzed birds that had at least 6 scored trials across all the tasks in a given year, based on the minimum number of data points needed to find a significant deviation from a binomial distribution with equal probabilities of two outcomes. In 2020-2021, this excluded 11 and 18 birds from initial and consistent bias datasets, respectively (Table A3.1). In 2019-20, this excluded 9 and 44 birds from initial and consistent bias datasets, respectively. Furthermore, 1 and $N = 1$ bird was removed as an outlier from each of the initial and consistent bias datasets for 2020-2021 (Table A3.1). For consistent bias data in 2020-2021, an additional 2819 trials across all birds and all cognitive tasks were excluded, in which birds moved different directions for their first and second movements but otherwise would have been analyzed in the analysis. Similarly, for consistent bias data in 2019-2020, an additional 497 trials across all birds and all cognitive tasks were excluded, in which birds moved different directions for their first and second movements but otherwise would have been analyzed in the analysis.

Statistical Analysis

To determine whether overall behavior was more biased than compared to chance, we used one-sample two-tailed t-tests to compare the sample mean to a theoretical mean if there was no directional bias in the sample ($LI = 0$). This test was used for initial and consistent bias measured across all trials and for only the first trials subset.

To determine whether individual birds showed significantly more directional bias compared to the sample mean, we calculated individual z-scores using bias estimates from all trials (Hopkins 2006). We considered z-scores ≤ -1.96 relatively right biased and z-scores $\geq +1.96$ relatively left biased, because there is a 95% chance of randomly selecting a value between -1.96 and + 1.96 (Wells 2003). Wald Chi-squared goodness-of-fit tests indicated whether the number of relatively left, right and unbiased trials across the entire sample differed significantly from chance. To determine whether different individuals demonstrated statistically significant directional bias, we conducted binomial tests for each individual using Bonferroni and Holm correction factors to reduce Type I error by correcting for multiple comparisons. As this was a conservative method, we reported original and corrected p-values in data uploaded to Mendeley Data repository (accessible at <http://dx.doi.org/10.17632/jsgv8jp8cp.2>).

To analyze variation in directional bias, linear models (LM) were fitted using four response variables: initial and consistent directional bias index for all trials and for the first trials of each task. For each of the bias index response variables, a separate LM was fitted for each predictor variable: elevation (categorical, high and low) and sex (categorical, male and female). This process was repeated with the strength alone of the bias index response variables.

To compare the initial and consistent bias indices measured across all trials, two LM were fitted with consistent bias index and strength as response variables and initial bias index and strength as predictor variables (respectively) between initial and consistent bias estimates.

Cognitive performance was estimated as the mean number of location errors per trial over the first 20 trials of the 2020-2021 spatial learning and memory task and the 2020-2021 single spatial reversal learning task following our previous work (Croston et al., 2017; Tello-Ramos et al., 2018; Sonnenberg et al., 2019; Heinen et al., 2021). Both metrics were used as response variables in LM with initial and consistent bias index and strength across all trials as predictors, and two other LM were fitted with initial and consistent bias strength (only) for the first trials. Fixed interaction effects of bias index estimates and elevation were also tested but were dropped from later analysis due to low explanatory value.

To estimate the individual repeatability of directional bias across all trials, repeatability analysis (with likelihood ratio test) was conducted with LI estimates from the main 2020-2021 analysis and from 2019-2020. We also fit linear mixed effects models (LMER) with LI as the response variable to explore how directional bias across all trials varied between years in only the birds that were observed in both years. “Individual” was held as a random slope.

To assess whether the first 2 visits of each trial were equally likely to be to feeders on the same side of the array versus on different sides, we conducted a one-sample t-test with the proportion of “same-side” visits compared to a predicted mean value of 0.5.

The maximum sample size available was used for each analysis. Sample sizes varied because we did not have sex or cognitive data for every bird, as not all birds were sexed conclusively, not all birds participated in both cognitive tasks and we needed fewer

minimum trials to calculate laterality (6 trials per year) than to calculate cognitive performance (20 trials per task).

Statistical Software

Analyses were performed using R version 4.1.0 (R Core Team 2018). LM were performed using the base stats package. LMER were performed using lme4 (Bates et al., 2015). Regression assumptions and goodness-of-fit were evaluated using DHARMA (Hartig, 2020) and the analysis of variance (ANOVA) test using car (Fox and Weisberg, 2019), reporting Wald Chi-squared values. The stats package was also used to calculate t-tests, reporting t-values (t), sample means (μ), and 95% confidence intervals (CI); and binomial tests, reporting Bonferroni-corrected p-values to reduce Type I error from multiple comparisons. The package rptR (Stoffel et al., 2017) was used to calculate repeatability (R), 95% CI, and goodness-of-fit based on a likelihood-ratio-test, additionally reporting deviance (D).

Results

Initial Directional Bias

In 2020-2021, 303 birds were scored for initial directional biases: 185 at high elevation and 118 at low elevation. Overall, chickadees showed a small significant initial bias to the left during search with a mean of 52% of left-scored trials across all cognitive tasks (mean LI = 2.92, one-sample t-test– $t = -36.05$, $df = 302$, $P < 0.001$, 95% CI =

[0.35, 5.49]; $N = 303$; Fig. 3.3A). Similarly, when only the first trial of each task was analyzed separately, chickadees also showed a small significant initial bias to the left, with a mean of 53% of left-scored trials (mean LI = 6.13, one-sample-t-test– $t = 3.23$, $df = 268$, $P = 0.001$, 95% CI = [2.39, 9.87], $N = 269$). For all scored trials, approximately 21% of birds ($N = 65$) were relatively more biased compared to the population mean ($-1.96 \leq z\text{-score} \leq 1.96$), which was significantly fewer birds than would be expected by chance if the sample contained equal numbers of initial biased and unbiased birds ($\chi^2 = 98.78$, $df = 1$, $P < 0.001$; $N = 303$). However, only 4 individuals showed a statistically significant directional bias compared to a binomial distribution (all left-biased), after correcting for repeated measures using a Bonferroni correction factor.

There was no significant difference between elevations in initial directional bias across all scored trials (measured through the laterality index; LM– $F_{1,301} = 0.056$, $P = 0.81$; $N = 303$; Fig. 3.3A) or only across the first trials of each task (LM– $b = 0.06$, $SE = 3.93$, $F_{1,267} < 0.001$, $P < 0.001$). Of the birds with sex data in 2020-2021, there was no significant effect of sex on initial directional bias across all trials (LM– $F_{1,128} = 0.58$, $P = 0.45$; $N = 130$) or in the first trials of each task (LM– $F_{1,114} = 0.02$, $P = 0.89$; $N = 116$).

Cognition and Initial Bias

Spatial learning and memory performance was not significantly associated with the initial bias index estimated from all scored trials with more than two location errors (LM: $b = -0.02$, $SE = 0.03$, $F_{1,229} = 0.74$, $P = 0.39$; $N = 231$; Fig. 3.4A), the strength alone of initial bias estimated using all trials (LM: strength: $b = 0.004$, $SE = 0.03$, $F_{1,229} = 0.02$,

$P = 0.89$; $N = 231$; Fig. 3.4C), or the strength alone of initial bias index estimated using only the first trial of each task (LM: $b = 0.02$, $SE = 0.03$, $F_{1,205} = 0.33$, $P = 0.56$, $N = 207$).

Single reversal learning performance was also not significantly associated with the initial bias index estimated from all scored trials with 2 or more location errors (LM: $b = -0.03$, $SE = 0.02$, $F_{1,206} = 2.85$, $P = 0.09$; $N = 208$; Fig. 3.5A), the strength alone of initial bias estimated for all scored trials (LM: strength: $b = 0.03$, $SE = 0.02$, $F_{1,206} = 2.42$, $P = 0.12$; $N = 208$; Fig. 3.5C), or the strength alone of initial bias estimated from first trials only (LM: $b = -0.01$, $SE = 0.02$, $F_{1,189} = 0.29$, $P = 0.59$; $N = 191$).

Consistent Directional Bias

There were 294 birds scored for consistent directional bias in 2020-2021, 182 at high elevation and 112 at low elevation. The initial and consistent directional bias indices were tightly correlated (Pearson's product-moment correlation test— correlation coefficient = 0.84, 95% CI: [0.81, 0.87], $t = 26.76$, $df = 292$, $P < 0.001$; Fig. 3.6A). The strength of initial and consistent directional bias was also significantly correlated, but less tightly than for the full directional bias indices (Pearson's product-moment correlation test— correlation coefficient = 0.65, 95% CI: [0.58, 0.71], $t = 14.51$, $df = 292$, $P < 0.001$; Fig. 3.6B).

For consistent bias, birds showed a small but significant preference to move towards the left than towards the right, with a mean of 53% of trials to the left (mean LI=

5.07, one sample t-test– 95% CI [1.42, 8.71], $t = 2.47$, $df = 293$, $P = 0.01$; $N = 294$; Fig. 3.3B). In the first trial of each task, before birds could learn the new feeder location, birds also showed a small but significant bias to move left, with a mean of 55% left-scored trials (mean LI = 9.65, one sample t-test– $t = 3.44$, $df = 188$, $P < 0.001$, 95% CI = [4.12, 15.18], $N = 189$). For all scored trials, approximately 22% of birds were relatively consistent biased ($N = 66$) compared to the sample mean ($-1.96 \leq z\text{-score} \leq 1.96$), which was significantly fewer birds than expected if the sample contained equal numbers of consistent biased and unbiased birds ($\chi^2 = 260.7$, $df = 2$, $p\text{-value} < 0.001$; $N = 294$). Of the 66 relatively consistent biased birds, the majority were left-biased ($N = 43$). Only three birds were statistically significantly biased according to a binomial test, all left-biased (after correction for multiple tests using the Bonferroni correction factor).

Consistent directional bias across all trials with two or more location errors did not vary across elevations (LM– $b = 3.20$, $SE = 3.81$, $F_{1,292} = 0.70$, $P = 0.40$; $N = 294$; Fig. 3.3B) or between sexes (LM– $b = -6.45$, $SE = 6.24$, $F_{1,122} = 1.07$, $P = 0.30$; $N = 124$). Consistent directional bias in the first trial of each task also did not vary across elevations (LM– $b = 4.04$, $SE = 5.91$, $F_{1,187} = 0.47$, $P = 0.49$; $N = 189$) or between sexes (LM– $b = -4.53$, $SE = 8.82$, $F_{1,84} = 0.26$, $P = 0.61$; $N = 86$).

Cognition and Consistent Directional Bias

Variation in spatial learning and memory performance was not significantly associated with differences in consistent directional biases for all scored trials with two or more location errors (LM– $b = -0.02$, $SE = 0.03$, $F_{1,223} = 0.34$, $P = 0.56$; $N = 225$; Fig.

3.4B), the strength alone of consistent biases for all scored trials (LM- $b = -0.01$, $SE = 0.03$, $F_{1,223} = 0.14$, $P = 0.71$; $N = 225$; Fig. 3.4D) or the strength alone of consistent biases for only the first trial of each task (LM- $b = -0.01$, $SE = 0.03$, $F_{1,156} = 0.12$, $P = 0.74$; $N = 158$).

Similarly, variation in single reversal learning performance was not associated with differences in consistent directional biases for all scored trials with at least 2 location errors (LM- $b = -0.02$, $SE = 0.02$, $F_{1,202} = 1.63$, $P = 0.20$; $N = 204$; Fig. 3.4B), or the strength alone of consistent directional biases using all scored trials (LM- $b = 0.03$, $SE = 0.02$, $F_{1,202} = 2.91$, $P = 0.09$; $N = 204$; Fig. 3.4D) or the strength alone of consistent biases using only the first trial of each task (LM- $b = 0.03$, $SE = 0.02$, $F_{1,143} = 1.56$, $P = 0.21$; $N = 145$).

Repeatability of Directional Bias Index

In 2019-2020, 206 birds were scored for initial directional bias and 170 for consistent bias. Between 2019-2020 and 2020-2021, directional bias scores were significantly repeatable at the individual level for both initial bias (repeatability: $R = 0.27$, 95% CI = [0.10, 0.43], $D = 8.57$, $df = 1$, $P < 0.001$; $N = 385$) and consistent bias (repeatability: $R = 0.22$, 95% CI = [0.04, 0.39], $D = 6.87$, $df = 1$, $P = 0.004$; $N = 366$). For only the subset of birds that were detected in both years, there was also no significant difference between years for initial (LMER: $b = -3.54$, $SE = 2.92$, $\chi^2 = 1.46$, $df = 1$, $P = 0.23$; $N = 124$) or consistent bias (LMER: $b = -2.03$, $SE = 3.74$, $\chi^2 = 0.38$, $df = 1$, $P = 0.54$; $N = 107$).

Same-Side Visits

Across all trials, birds were equally likely to visit the feeder on the same side of the array as a feeder on an adjacent side, with 51% of trials to the same side feeder (one-sample t-test: $t = 1.56$, $df = 302$, $P = 0.12$, 95% CI = [0.50, 0.52], $N = 303$; Fig. 3.7).

Discussion

Overall, we found that chickadees did not show directional bias in movement around the feeder arrays at either high or low elevation when learning a spatial task: although there was a small but significant bias to the left for both initial and consistent biases, this bias was small, and the majority of birds chose to move left and right relatively equally while searching for a food reward. Search behavior was targeted in the analysis by using trials in which birds visited at least two unrewarding feeders before visiting the rewarding feeder (e.g., two or more location errors), so that birds had not yet learned the location of the rewarding feeder during the spatial cognitive tasks. However, the results were the same even if we only used the first trial of each task before the birds even located the rewarding feeder. Few birds showed significant directional biases, although there was notable individual variation. There were no significant trends in directional bias scores between elevations or sexes, suggesting that movement around the arrays was not significantly influenced by environmental harshness or social dominance. Furthermore, neither variation in performance on the spatial learning and memory task

nor in performance in the single spatial reversal learning task was significantly associated with differences in directional biases or in the strength of directional biases regardless of whether we used all trials in which birds visited at least two unrewarding feeders or only the first trial for each task. These null results suggest that estimates of spatial cognitive performance measured using this experimental apparatus did not appear to be significantly biased by the direction birds chose to move around the feeder array. In addition, these results also show that the learning process does not affect the movement bias during all trials with two or more location errors.

The directional bias index was mildly and significantly repeatable for both initial and consistent bias. This suggests that although individuals were not strongly biased to the left or right, birds behaved similarly while searching for a food reward between two consecutive winter seasons with most birds having no bias. This may suggest that birds have somewhat stable search strategies or general movement preferences at the feeder arrays, but that this behavior was not well-described by passive directional biases estimated using the bias index. Furthermore, there was no significant difference in directional bias estimates between two consecutive years, for birds detected during both years. This further suggests that the individual variation reported in our study did not appear to vary across the sample with annual conditions.

These null results suggest that chickadees do not demonstrate directional biases in movement during search when learning a spatial task, and thus existing lateralization may not affect decision-making related to movement direction while searching the feeder arrays. However, as our study is observational and does not directly test for lateralization,

we can only speculate and it is also possible that any existing lateralization simply does not affect the decision process during movement while searching for food. Lateralization has been well documented in avian species (Rogers, 2021), including in spatial cognition (Clayton and Krebs, 1993; Güntürkün et al., 2020) and associated brain regions (Siegel et al., 2006; Jonckers et al., 2015). However, motor-based laterality may not directly correlate with lateralization; rather, it may only indicate that one hemisphere dominates the control of a given behavior (Rogers, 2021). If neither hemisphere dominates a chickadee's decision to move left or right after approaching the feeder array, then lateralization may not be detected through movement around the array. Furthermore, lateralization appears to be task dependent (Schiffner and Srinivasan, 2013; Karenina and Giljov, 2022; Penry-Williams et al., 2022). Thus, it is possible that birds might not demonstrate laterality while searching for a food reward without additional cognitive demands, such as predator vigilance (Piddington and Rogers, 2013). We did not have the data to explore this alternative.

Other noncognitive factors may have affected the direction chickadees chose to move around the feeder array, obscuring possible lateralization that may have been present. For example, socially subordinate birds may have been displaced from preferred feeders by more dominant birds, leading to more random directional bias. However, we find this unlikely because we found no significant difference in either initial or consistent directional bias between sexes for all trials and first trials alone, despite known differences in social dominance between males and females in Parid species (Ekman, 1989). This is consistent with our previous work, which shows that social dominance rank did not significantly affect learning (i.e., number of errors; Heinen et al., 2021). It is

possible that we did not see effects of dominance or displacement because we only used trials with at least two consecutive location errors, and so only analyzed trials in which birds consistently moved in the same direction without being displaced. Such random disruption might have been more prominent in our dataset if we included trials with only one location error, or if we included trials in which birds moved across the feeder array. In addition, it is unlikely that a bird would be displaced multiple times while consistently moving in the same direction across the feeders that do not provide food.

Our results do not support previous literature suggesting that more strongly lateralized individuals perform better on spatial cognitive tasks (Rogers, 2021). However, our findings are not completely unexpected and contribute to a body of work reporting null or negative associations between behavioral asymmetries and cognitive performance (e.g., Whiteside et al., 2020). It is possible that our findings are simply due to the lack of lateralization in foraging-based search behavior in chickadees, and that by measuring a different behavior we might find the expected relationship between directional biases and cognitive ability. This might be likely if the cognitive processes and structures involved in foraging search behavior differ from those involved in spatial learning and memory. To assess this, further research involving several lateralized behaviors across multiple types of cognitive tasks (i.e., spatial learning, visual discrimination, etc.) would be needed. Another explanation could be that directional biases may be costly in natural conditions due to environmental factors. To speculate, directional bias in movement could result in poorer foraging efficiency, or less efficient cache retrieval. Such directional bias may be more costly in harsh environments in which food may be less predictable or metabolic requirements may be higher, and thus could be less expressed in

our study system. If this were the case, we might expect directional bias to vary seasonally or vary across elevations that differ significantly in environmental harshness. However, we did not find the latter. And although we do not have the data to test for seasonal variation in directional biases, we have found that variation in spatial learning and memory has a strong genetic component (Branch et al., 2022). Finally, we speculate that lateralization in food-caching species may be constrained by other aspects of cognition that may be more advantageous, such as memory transfer or memory capacity. As food-caching species, chickadees rely on spatial learning and memory ability for cache retrieval, and this cognitive ability appears to be under selection in our birds (Sonnenberg et al., 2019). But the benefits of lateralization for food-caching are still unclear and may depend on the ability to transfer information from one side of the brain to the other (Clayton and Krebs, 1993). While more research is needed, this could explain why directional biases were not evident in the spatial cognitive tasks used in this study.

Finally, we must consider several limitations of the study and the possible effects on our interpretations. First, although feeders were relatively equidistant from each other, the square shape of the feeder array could bias birds to move more frequently between feeders on the same side of the square array. However, birds were equally likely to move to another feeder on the same side of the array as to a feeder on one of the adjacent sides after making a location error. Second, we used visit data to infer movements between feeders, but did not validate these data through behavioral observations. As such, we do not know how frequently birds might have left the arrays in between feeder visits, leading us to misinterpret those trials for left or right bias. However, we have often observed that birds do not leave the arrays until they have found a food reward, typically sampling

feeders consecutively until they reach the correct feeder (see Supplemental Video). In addition, the mean time for trials with at least two location errors was small (<1 min), suggesting that visits were in rapid succession. This suggests that birds likely did not move away from the array during these trials, considering that all nearby foliage was ca. 3m away and so the only nearby perches that were not attached to the feeders were the wires that suspended each array. Plus, consistent bias estimates should be more robust to this type of bias than initial bias since consistent bias was calculated from two consecutive pairs of visit data. Yet, both initial and consistent directional bias estimates yielded similar results in our analyses, suggesting that both bias estimates used visit data that represented actual movements around the feeder array. Third, in our study, we estimated directional bias while birds were learning a spatial task, specifically using trials with 2+ or more location errors so that birds had not fully learned the feeder location yet. Thus, there is a possibility that learning itself might have affected the birds' movement rather than passive directional biases. We find this unlikely because if learning affected movement bias, then we would expect to see an association between cognitive ability and directional biases, which we do not. We also found similar results whether we used all qualifying trials from the cognitive tasks or only the first trials of each task, in which birds had not found the correct rewarding feeder yet and thus should be free from a possible confounding effect of learning. Finally, our study can only address movement bias, not lateralization. While directional bias might be very similar, the feeder perches allow birds to use either eye to look either direction before deciding to move to another feeder perch. Thus, our setup is appropriate to evaluate how directional biases might

affect decision-making during a cognitive task. But they should not be used to assume that these movements are driven by underlying lateralization at a neural level.

A major challenge in cognitive ecology is designing experiments that test specific cognitive traits and minimize the role of other non-cognitive factors. Assessing how directional biases might impact behavior during cognitive tasks is thus essential to interpret cognitive performance, especially when experiments are conducted in natural settings. Our null results from a large sample size suggest that mountain chickadees in our system did not demonstrate strong left or right preferences in movement while searching for a food reward, before learning the location of a rewarding feeder during spatial cognitive tasks. These results also show that individual variation in these directional biases did not affect performance on two cognitive tasks. Directional biases may still be present in our population and may be more apparent in other tasks or behaviors; but more research is needed to understand how such biases might be important for spatial cognitive tasks in natural contexts.

Overall, we did not detect significant directional biases in the foraging behavior of food-caching mountain chickadees across two consecutive winters in two different environments characterized by strong differences in winter conditions. Our results show that search behavior in food-caching chickadees did not appear to be biased by individual preferences in movement-related decision-making, which might allow more efficient search during foraging.

Acknowledgements

This work was supported by the NSF IOS1856181 and IOS2119824 to VVP. LMB and BRS were supported by the NSF Graduate Research Fellowship Program. Thank you to the staff of Sagehen Experimental Forest and Sagehen Creek Field Station (University of California Berkeley) for assistance. We also thank Carrie L. Branch and Angela M. Pitera for feedback during the writing process. Constructive comments from two reviewers significantly improved the manuscript. We particularly appreciate thought-provoking and constructive comments from Dr. Arnon Lotem, which forced us to think critically about our data and interpretation of our results.

Author Contributions

Lauren M. Benedict: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original draft preparation, Writing – Reviewing and editing, Visualization.

Virginia K. Heinen: Methodology, Investigation, Data curation, Writing – Reviewing & editing. **Benjamin R. Sonnenberg:** Investigation, Writing – Reviewing & editing. **Eli S.**

Bridge: Methodology, Writing- Review & editing. **Vladimir V. Pravosudov:** Conceptualization, Methodology, Investigation, Writing – Original draft preparation, Writing – Reviewing and editing, Funding acquisition, Project administration, Supervision.

Ethics Approval

The use of animals adhered to the guidelines set forth by the University of Nevada Reno Institutional Care and Use Committee (Protocol 00818, 00046 and 00603) and the California Department of Fish and Wildlife (Permit D-0011776516-4). Banding wild birds adhered to guidelines set forth by the U.S. Geological Survey Bird Banding Laboratory (Federal Bird Banding Permit 22878). To the best of our knowledge, no birds were harmed by the collection of these data and birds were only handled for a few minutes during banding. We detected no negative effects of using PIT-tags and color bands during our study.

Data Availability

The datasets generated during and/or analyzed during the current study are available in the Mendeley Data repository, <http://dx.doi.org/10.17632/jsgv8jp8cp.2>

References

- Bates D, Machler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bell ATA, Niven JE (2016) Strength of forelimb lateralization predicts motor errors in an insect. *Biology Letters*, 12:20160547. <https://doi.org/10.1098/rsbl.2016.0547>
- Benedict LM, Pitera AM, Branch CL, Sonnenberg BR, Heinen VK, Bridge ES, Pravosudov VV (2021) Information maintenance of food sources is associated with environment, spatial cognition and age in a food-caching bird. *Animal Behaviour*, 182:153–172. <https://doi.org/10.1016/j.anbehav.2021.10.009>
- Bisazza A, Cantalupo C, Capocchiano M, Vallortigara G (2000) Population lateralisation and social behaviour: A study with 16 species of fish. *Laterality*, 5:269–284. <https://doi.org/10.1080/713754381>
- Bisazza A, Cantalupo C, Robins A, Rogers LJ, Vallortigara G (1996) Right-pawedness in toads. *Nature*, 379:408–408. <https://doi.org/10.1038/379408a0>
- Bisazza A, Facchin L, Pignatti R, Vallortigara G (1998a) Lateralization of detour behaviour in poeciliid fish: The effect of species, gender and sexual motivation. *Behavioral Brain Research*, 91:157–164. [https://doi.org/10.1016/S0166-4328\(97\)00114-9](https://doi.org/10.1016/S0166-4328(97)00114-9)
- Bisazza A, Rogers LJ, Vallortigara G (1998b) The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience and Biobehavioral Reviews*, 22:411–426. [https://doi.org/10.1016/S0149-7634\(97\)00050-X](https://doi.org/10.1016/S0149-7634(97)00050-X)
- Bisazza A, Sovrano VA, Vallortigara G (2001) Consistency among different tasks of left–right asymmetries in lines of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia*, 39:1077–1085. [https://doi.org/10.1016/S0028-3932\(01\)00034-3](https://doi.org/10.1016/S0028-3932(01)00034-3)
- Branch CL, Semenov GA, Wagner DN, Sonnenberg BR, Pitera AM, Bridge ES, Taylor SA, Pravosudov VV et al (2022) The genetic basis of spatial cognitive variation in a food-caching bird. *Current Biology*, 32:210–219.e4. <https://doi.org/10.1016/j.cub.2021.10.036>
- Bridge ES, Wilhelm J, Pandit M et al (2019) An Arduino-based RFID platform for animal research. *Frontiers in Ecology and Evolution*, 7:257. <https://doi.org/10.3389/fevo.2019.00257>

- Brown C, Magat M (2011) Cerebral lateralization determines hand preferences in Australian parrots. *Biology Letters*, 7:496–498.
<https://doi.org/10.1098/rsbl.2010.1121>
- Clayton NS, Krebs JR (1993) Lateralization in Paridae: comparison of a storing and a non-storing species on a one-trial associative memory task. *Journal of Comparative Physiology A*, 171:807–815. <https://doi.org/10.1007/BF00213077>
- Clayton NS, Krebs JR (1994) Lateralization and unilateral transfer of spatial memory in marsh tits: are two eyes better than one? *Journal of Comparative Physiology A*, 174:769–773. <https://doi.org/10.1007/BF00192726>
- Croston R, Branch CL, Pitera AM, Kozlovsky DY, Bridge ES, Parchman T, Pravosudov VV (2017) Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123:139–149. <https://doi.org/10.1016/j.anbehav.2016.10.004>
- Croston R, Kozlovsky DY, Branch CL, Parchman TL, Bridge ES, Pravosudov VV (2016) Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behaviour*, 111:225–234.
<https://doi.org/10.1016/j.anbehav.2015.10.015>
- Dadda M, Agrillo C, Bisazza A, Brown C (2015) Laterality enhances numerical skills in the guppy, *Poecilia reticulata*. *Frontiers in Behavioral Neuroscience*, 9:285.
<https://doi.org/10.3389/fnbeh.2015.00285>
- Dadda M, Koolhaas WH, Domenici P (2010) Behavioural asymmetry affects escape performance in a teleost fish. *Biology Letters*, 6:414–417.
<https://doi.org/10.1098/rsbl.2009.0904>
- Dadda M, Zandonà E, Agrillo C, Bisazza A (2009) The costs of hemispheric specialization in a fish. *Proceedings of the Royal Society B: Biological Sciences*, 276:4399–4407. <https://doi.org/10.1098/rspb.2009.1406>
- Ekman J (1989) Ecology of non-breeding social systems of Parus. *Wilson Bulletin*, 101:263–288
- Ferrari MCO, McCormick MI, Mitchell MD, Allan BJM, Gonçalves EJ, Chivers DP (2017) Daily variation in behavioural lateralization is linked to predation stress in a coral reef fish. *Animal Behaviour*, 133:189–193.
<https://doi.org/10.1016/j.anbehav.2017.09.020>
- Fox J, Weisberg S (2019) *An R Companion to Applied Regression*, 3rd edn. Sage, Thousand Oaks, CA

- Freas CA, LaDage LD, Roth TC II, Pravosudov VV (2012) Elevation-related differences in memory and the hippocampus in mountain chickadees, *Poecile gambeli*. *Animal Behaviour*, 84:121–127. <https://doi.org/10.1016/j.anbehav.2012.04.018>
- Gawel K, Gibula E, Marszalek-Grabska M, Filarowska J, Kotlinska JH (2019) Assessment of spatial learning and memory in the Barnes maze task in rodents—methodological consideration. *Naunyn-Schmiedeberg's Arch Pharmacology*, 392:1–18. <https://doi.org/10.1007/s00210-018-1589-y>
- Güntürkün O, Ströckens F, Ocklenburg S (2020) Brain lateralization: a comparative perspective. *Physiology Reviews*, 100:1019–1063. <https://doi.org/10.1152/physrev.00006.2019>
- Hartig F (2020) DHARMA: residual diagnostics for hierarchical (multi-level / mixed) regression models. Version 0.2.7, <https://CRAN.R-project.org/package=DHARMA>
- Heinen VK, Benedict LM, Pitera AM, Sonnenberg BR, Bridge ES, Pravosudov VV (2021) Social dominance has limited effects on spatial cognition in a wild food-caching bird. *Proceedings of the Royal Society B: Biological Sciences*, 288:20211784. <https://doi.org/10.1098/rspb.2021.1784>
- Hopkins WD (2006) Comparative and familial analysis of handedness in great apes. *Psychological Bulletin*, 132:538–559. <https://doi.org/10.1037/0033-2909.132.4.538>
- Hopkins WD, Phillips KA, Bania A, Calcutt SE, Gardner M, Russell J, Schaeffer J, Lonsdorf EV, Ross SR, Schapiro SJ (2011) Hand preferences for coordinated bimanual actions in 777 great apes: Implications for the evolution of handedness in Hominins. *Journal of Human Evolution*, 60:605–611. <https://doi.org/10.1016/j.jhevol.2010.12.008>
- Isparta S, Salgirli Demirbas Y, Bars Z, Cinar Kul B, Güntürkün O, Ocklenburg S, Da Graca Pereira G (2020) The relationship between problem-solving ability and laterality in cats. *Behavioural Brain Research*, 391:112691. <https://doi.org/10.1016/j.bbr.2020.112691>
- Jonckers E, Güntürkün O, De Groof G, Van der Linden A, Bingman VP (2015) Network structure of functional hippocampal lateralization in birds. *Hippocampus*, 25:1418–1428. <https://doi.org/10.1002/hipo.22462>
- Jozet-Alves C, Viblanc VA, Romagny S, Dacher M, Healy SD, Dickel L (2012) Visual lateralization is task and age dependent in cuttlefish, *Sepia officinalis*. *Animal Behaviour*, 83:1313–1318. <https://doi.org/10.1016/j.anbehav.2012.02.023>

- Karenina K, Giljov A (2022) Lateralization in feeding is food type specific and impacts feeding success in wild birds. *Ecology and Evolution*, 12:e8598. <https://doi.org/10.1002/ece3.8598>
- Kozlovsky DY, Branch CL, Pitera AM, Pravosudov VV (2018) Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *Royal Society Open Science*, 5:171604. <https://doi.org/10.1098/rsos.171604>
- Leaver LA, Ford S, Miller CW, Yeo MK, Fawcett TW (2020) Learning is negatively associated with strength of left/right paw preference in wild grey squirrels (*Sciurus carolinensis*). *Learning and Behavior*, 48:96–103. <https://doi.org/10.3758/s13420-019-00408-2>
- Letzkus P, Boeddeker N, Wood JT, Zhang S-W, Srinivasan MV (2008) Lateralization of visual learning in the honeybee. *Biology Letters*, 4:16–19. <https://doi.org/10.1098/rsbl.2007.0466>
- Loconsole M, Mascalzoni E, Daisley JN, De Agrò M, Vallortigara G, Regolin L (2021) Lateralized declarative-like memory for conditional spatial information in domestic chicks (*Gallus gallus*). *Symmetry*, 13:906. <https://doi.org/10.3390/sym13050906>
- Magat M, Brown C (2009) Laterality enhances cognition in Australian parrots. *Proceedings of the Royal Society B: Biological Sciences*, 276:4155–4162. <https://doi.org/10.1098/rspb.2009.1397>
- Meigs JB, Smith DC, Van Buskirk J (1983) Age determination of black-capped chickadees. *Journal of Field Ornithology*, 54:283–286
- Miler K, Kuszewska K, Woyciechowski M (2017) Larval antlions with more pronounced behavioural asymmetry show enhanced cognitive skills. *Biology Letters*, 13:20160786. <https://doi.org/10.1098/rsbl.2016.0786>
- Penry-Williams IL, Brown C, Ioannou CC (2022) Detecting behavioural lateralisation in *Poecilia reticulata* is strongly dependent on experimental design. *Behavioral Ecology and Sociobiology*, 76:25. <https://doi.org/10.1007/s00265-022-03135-3>
- Piddington T, Rogers LJ (2013) Strength of hand preference and dual task performance by common marmosets. *Animal Cognition*, 16:127–135. <https://doi.org/10.1007/s10071-012-0562-2>
- Pitera AM, Branch CL, Bridge ES, Pravosudov VV (2018) Daily foraging routines in food-caching mountain chickadees are associated with variation in environmental harshness. *Animal Behaviour*, 143:93–104. <https://doi.org/10.1016/j.anbehav.2018.07.011>

- Pyle P (1997) Molt limits in North American passerines. *North American Bird Bander*, 22:49–89
- R Core Team (2018) R: a language and environment for statistical computing, version 3.5.2. R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>
- Robins A, Rogers LJ (2004) Lateralized prey-catching responses in the cane toad, *Bufo marinus*: analysis of complex visual stimuli. *Animal Behaviour*, 68:767–775. <https://doi.org/10.1016/j.anbehav.2003.12.014>
- Rogers LJ (2021) Brain lateralization and cognitive capacity. *Animals*, 11:1996. <https://doi.org/10.3390/ani11071996>
- Rogers LJ, Vallortigara G, Andrew RJ (2013) Divided brains: the biology and behaviour of brain asymmetries. Cambridge University Press, Cambridge, UK
- Rogers LJ, Zucca P, Vallortigara G (2004) Advantages of having a lateralized brain. *Proceedings of the Royal Society B: Biological Sciences*, 271:S420–S422. <https://doi.org/10.1098/rsbl.2004.0200>
- Scharf HM, Stenstrom K, Dainson M, Benson TJ, Fernandez-Juricic E, Hauber ME (2019) Mimicry-dependent lateralization in the visual inspection of foreign eggs by American robins. *Biology Letters*, 15:20190351. <https://doi.org/10.1098/rsbl.2019.0351>
- Schiffner I, Srinivasan MV (2013) Behavioural lateralization in budgerigars varies with the task and the individual. *PLoS ONE*, 8:e82670. <https://doi.org/10.1371/journal.pone.0082670>
- Shen J, Fang K, Fan Y, Song J, Yang J, Shen D, Liu Y, Fang G (2019) Dynamics of electroencephalogram oscillations underlie right-eye preferences in predatory behavior of the music frogs. *Journal of Experimental Biology*, jeb.212175. <https://doi.org/10.1242/jeb.212175>
- Siegel JJ, Nitz D, Bingman VP (2006) Lateralized functional components of spatial cognition in the avian hippocampal formation: Evidence from single-unit recordings in freely moving homing pigeons. *Hippocampus*, 16:125–140. <https://doi.org/10.1002/hipo.20139>
- Sonnenberg BR, Branch CL, Pitera AM, Bridge ES, Pravosudov VV (2019) Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, 29:670–676. <https://doi.org/10.1016/j.cub.2019.01.006>
- Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8:1639–1644. <https://doi.org/10.1111/2041-210X.12797>

- Tello-Ramos MC, Branch CL, Pitera AM, Kozlovsky DY, Pravosudov VV (2018) Memory in wild mountain chickadees from different elevations: comparing first-year birds with older survivors. *Animal Behaviour*, 137:149-160. <https://doi.org/10.1016/j.anbehav.2017.12.019>
- Tommasi L, Andrew RJ, Vallortigara G (2000) Eye use in search is determined by the nature of task in the domestic chick (*Gallus gallus*). *Behavioural Brain Research*, 112:119–126. [https://doi.org/10.1016/S0166-4328\(00\)00167-4](https://doi.org/10.1016/S0166-4328(00)00167-4)
- Tommasi L, Gagliardo A, Andrew RJ, Vallortigara G (2003) Separate processing mechanisms for encoding of geometric and landmark information in the avian hippocampus: Geometric information in the avian hippocampus. *European Journal of Neuroscience*, 17:1695–1702. <https://doi.org/10.1046/j.1460-9568.2003.02593.x>
- Ventolini N, Ferrero EA, Sponza S, Della Chiesa A, Zucca P, Vallortigara G (2005) Laterality in the wild: preferential hemifield use during predatory and sexual behaviour in the black-winged stilt. *Animal Behaviour*, 69:1077–1084. <https://doi.org/10.1016/j.anbehav.2004.09.003>
- Wells DL (2003) Lateralised behaviour in the domestic dog, *Canis familiaris*. *Behavioral Processes*, 61:27–35. [https://doi.org/10.1016/S0376-6357\(02\)00161-4](https://doi.org/10.1016/S0376-6357(02)00161-4)
- Whiteside MA, Bess MM, Frasnelli E, Beardsworth CE, Langley EJG, van Horik JO, Madden JR (2018) Low survival of strongly footed pheasants may explain constraints on lateralization. *Science Reports*, 8:13791. <https://doi.org/10.1038/s41598-018-32066-1>
- Whiteside MA, Bess MM, Frasnelli E, Beardsworth CE, Langley EJG, van Horik JO, Madden JR (2020) No evidence that footedness in pheasants influences cognitive performance in tasks assessing colour discrimination and spatial ability. *Learning and Behavior*, 48:84–95. <https://doi.org/10.3758/s13420-019-00402-8>
- Zhao D, Hopkins WD, Li B (2012) Handedness in nature: First evidence on manual laterality on bimanual coordinated tube task in wild primates. *American Journal of Physical Anthropology*, 148:36–44. <https://doi.org/10.1002/ajpa.22038>
- Zucca P, Sovrano VA (2008) Animal lateralization and social recognition: Quails use their left visual hemifield when approaching a companion and their right visual hemifield when approaching a stranger. *Cortex*, 44:13–20. <https://doi.org/10.1016/j.cortex.2006.01.002>

Tables

Table 3.1

Term	Definition or usage
Lateralization	Asymmetric structure and function of the brain and nervous system (Rogers et al. 2013). Also called cerebral or hemispheric specialization.
Directional bias	Consistent behavior using one visual field, side of the body, limb, etc. Distinct from lateralization because directional biases might arise from various processes, not necessarily specialized structure or function in the brain that would indicate lateralization. Also called ‘directional preferences’ or ‘asymmetric behavior’.
Laterality	For this study, used to refer to asymmetry in behavior that is likely related to lateralization, or has been shown to relate to lateralization. Whereas lateralization ultimately refers to the asymmetric neural structure or function between two sides of the brain and nervous system, laterality typically characterizes asymmetric behaviors or consistent directional biases that likely derive from lateralization.
Strength of directional bias	Regardless of direction, the frequency or consistency of behaviors to one specific side (left or right). In this study, birds that move left and right relatively equally would have weak directional bias whereas birds that always move to the left or always move to the right would have strong directional bias. Similar to strength of laterality.
Search behavior	When birds visit feeders at the feeder array at the beginning of a cognitive task, before learning the location of the rewarding feeder. If the bird makes at least 2 location errors in a trial, this suggests that the bird is searching for an unknown reward location.
Trial	Begins when a bird visits a feeder at the array during a spatial cognitive task and ends when the bird finds the correct rewarding feeder and receives a food reward.
Location errors	A visit to any unrewarding feeder location at the feeder arrays during spatial cognitive tasks
Spatial learning and memory performance	Performance on an associative learning task in which birds must learn one rewarding location out of 8 locations in a spatial array. Estimated using the mean number of location errors per trial in the first 20 trials of the spatial learning and memory task (Croston et al. 2016, 2017)
Single reversal learning performance	Performance on a reversal learning task with a single reversal. After birds learn an association between a rewarding location and a food reward, the task is ‘reversed’ by assigning birds to a different feeder in the feeder array and measuring learning performance for the new feeder. Estimated using the mean number of location errors per trial in the first 20 trials of the single reversal learning task (Croston et al. 2017).
Serial reversal learning task	Similar to the single reversal learning task, but after an initial learning period, the rewarding feeder location is alternated between two different locations every day.

Figures

Figure 3.1

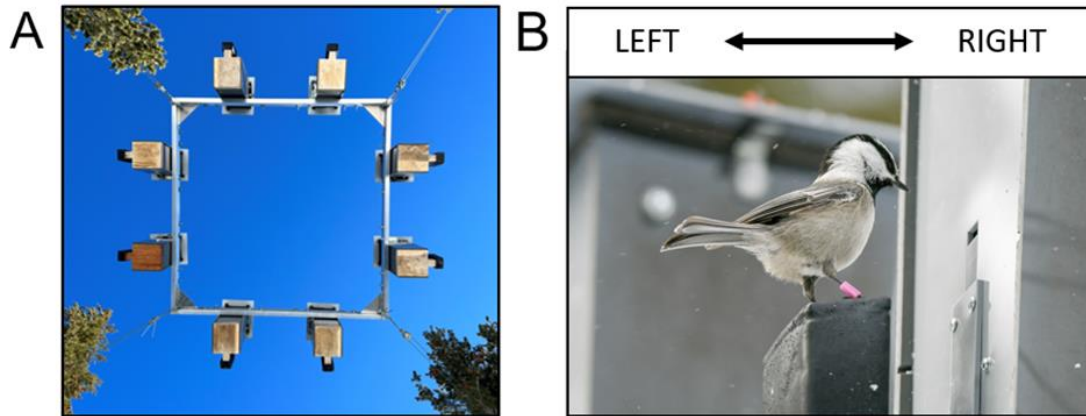


Figure 3.1. Experimental apparatus. (A) One of four feeder arrays, viewed from the ground looking upwards. Eight feeders (wooden squares) with protruding perches (black squares) are equidistantly arranged on a 122×122 cm square aluminum frame with 2 feeders per side. The array is elevated ca. 3m in the air (depending on winter snow level) and is ca. > 3 m away from trees on all other sides. (B) A PIT-tagged bird (pink tag) lands on the perch of a feeder of an unrewarding feeder. The mechanized feeder door does not open, and the bird does not receive a food reward. The bird can choose to move left or right to visit another nearby feeder, indicated by arrows above the image

Figure 3.2

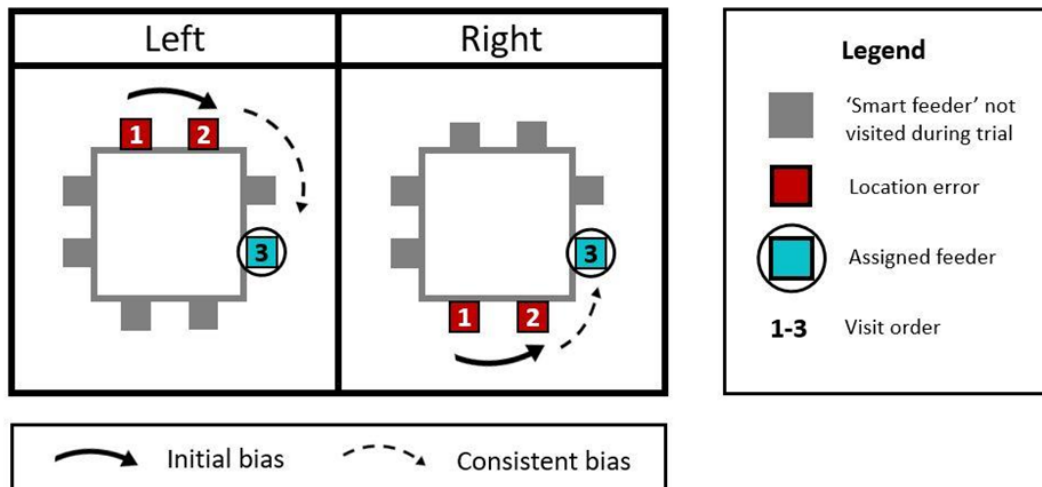


Figure 3.2. Scoring trial direction. Trials were scored for initial bias based on the relative position of the second location error compared to the first location error: left and right . Similarly, consistent bias was scored for trials in which the position of the third visit was in the same direction as the initial bias. Trials were only scored if the feeder locations were within two feeders apart and if the bird made at least two location errors, indicating that birds had not yet learned the location of the rewarding feeder location. Note that this figure depicts a ‘top-down’ depiction of the feeder arrays, in contrast to Fig. 3.1

Figure 3.3

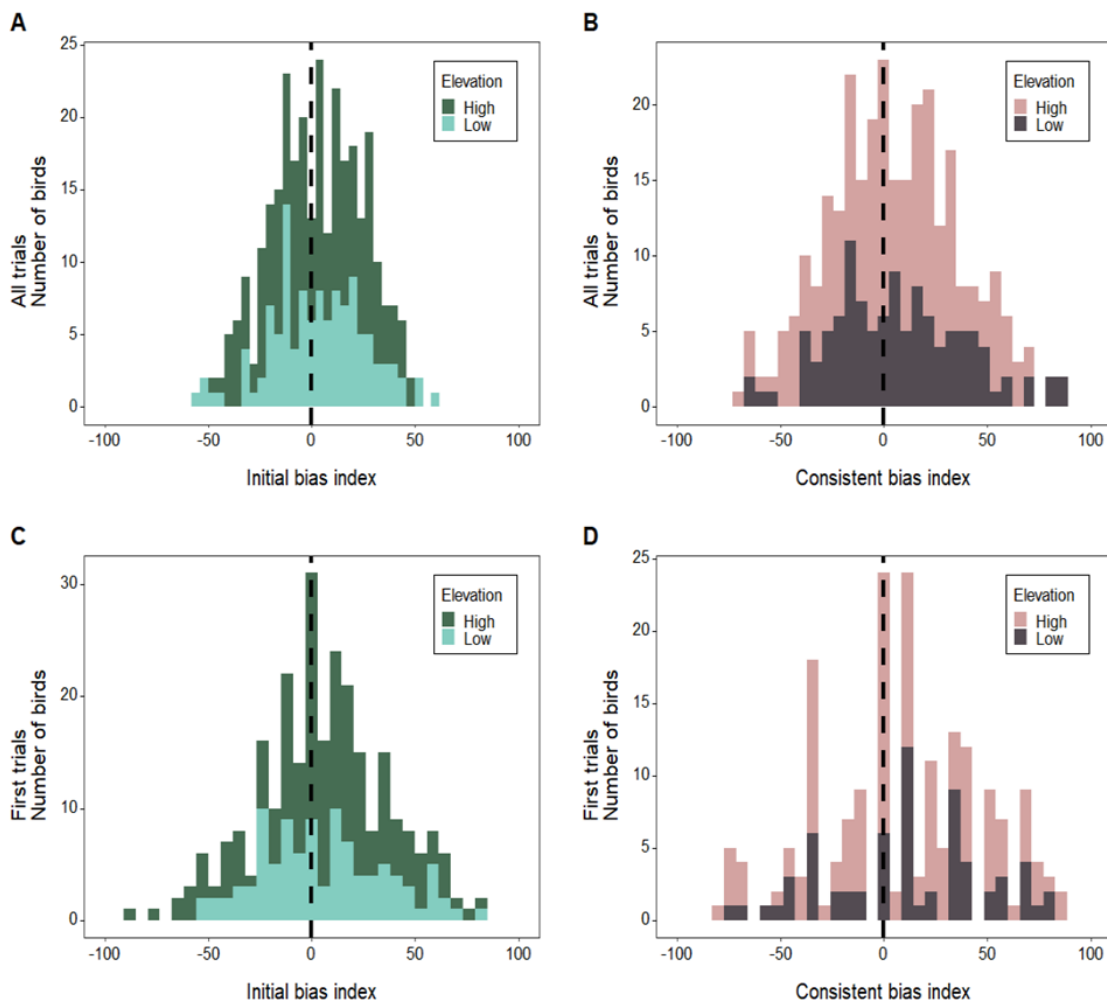


Figure 3.3. Distribution of (A, C) initial and (B, D) consistent bias index scores for all trials in which birds visited at least two unrewarding feeders (e.g., two location errors) (A, B) and for the first trial of each task only (C, D) by elevation. The directional bias index ranges from fully right-biased (LI = -100) to fully left-biased (LI = 100) with middle values indicating unbiased (LI = 0, dashed line). Elevations are stacked (as in, low + high = total frequency). For (A), N = 303, for (B), N = 294, for (C) N = 269, and for (D) N = 189.

Figure 3.4

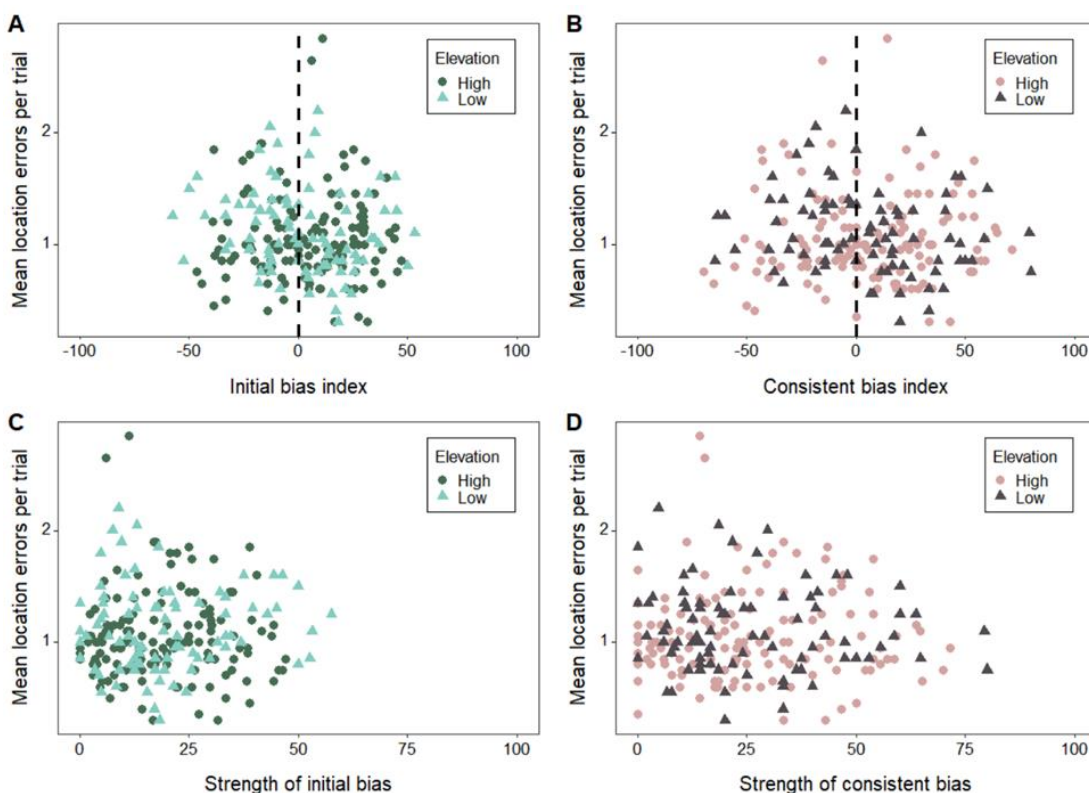


Figure 3.4. Spatial learning and memory performance by (A) initial bias, (B) consistent bias, (C) strength of initial bias, and (D) strength of consistent bias. All bias estimates used all trials with at least 2 location errors. Cognitive performance measured in mean location errors per trial in the first 20 trials of the cognitive task, with smaller values indicative of better performance. Directional bias index ranges from fully right-biased (LI = -100) to fully left-biased (LI = 100) and the strength ranges from no bias (strength = 0) to fully biased (strength = 100) regardless of direction. For (A) and (C), $N = 229$. For (B) and (D), $N = 225$

Figure 3.5

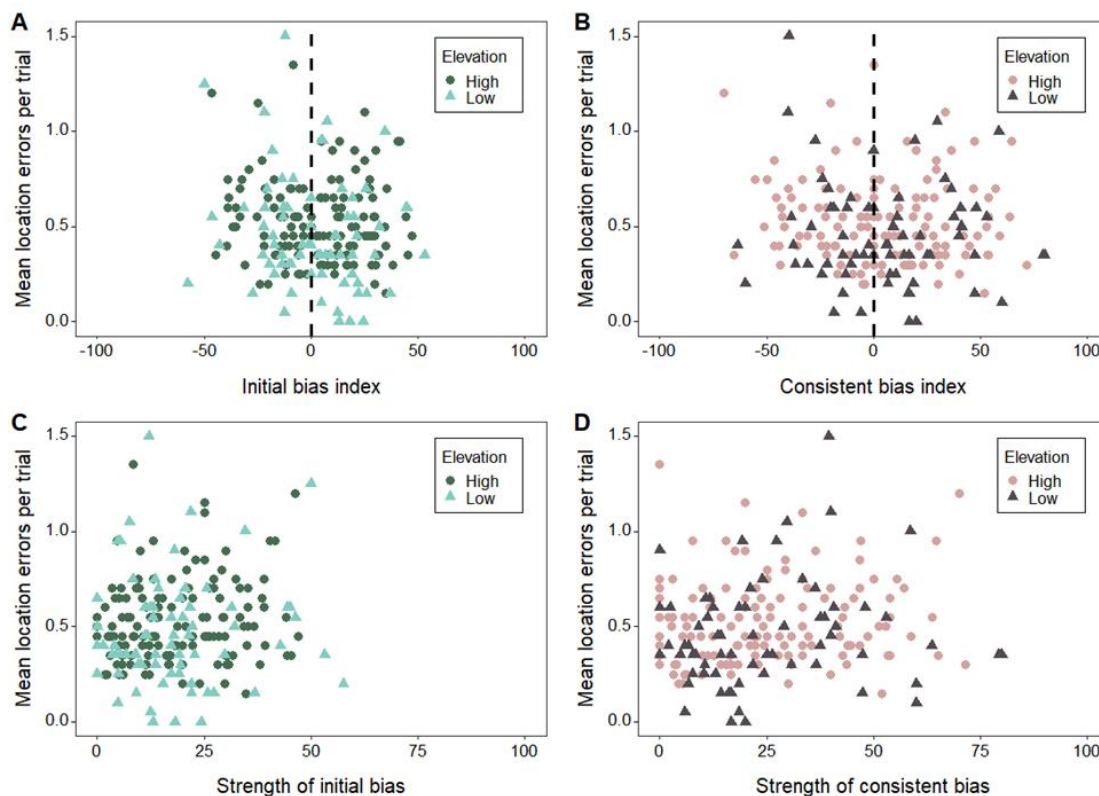


Figure 3.5. Single reversal learning performance by (A, C) initial and (B, D) consistent directional bias (A, C) index and (C, D) strength. All bias estimates used all trials with at least 2 location errors. Cognitive performance measured in mean location errors per trial in the first 20 trials of the cognitive task, with smaller values indicative of better performance. Directional bias index ranges from fully right-biased (LI = -100) to fully left-biased (LI = 100) and the strength ranges from no bias (strength = 0) to fully biased (strength = 100) regardless of direction. Initial directional bias (greens) and consistent directional bias (red and purple) shown by high (green and red circles) and low (light green and purple triangles) elevation, even though elevation was not a focal variable. For (A) and (C), $N = 208$. For (B) and (D), $N = 204$.

Figure 3.6

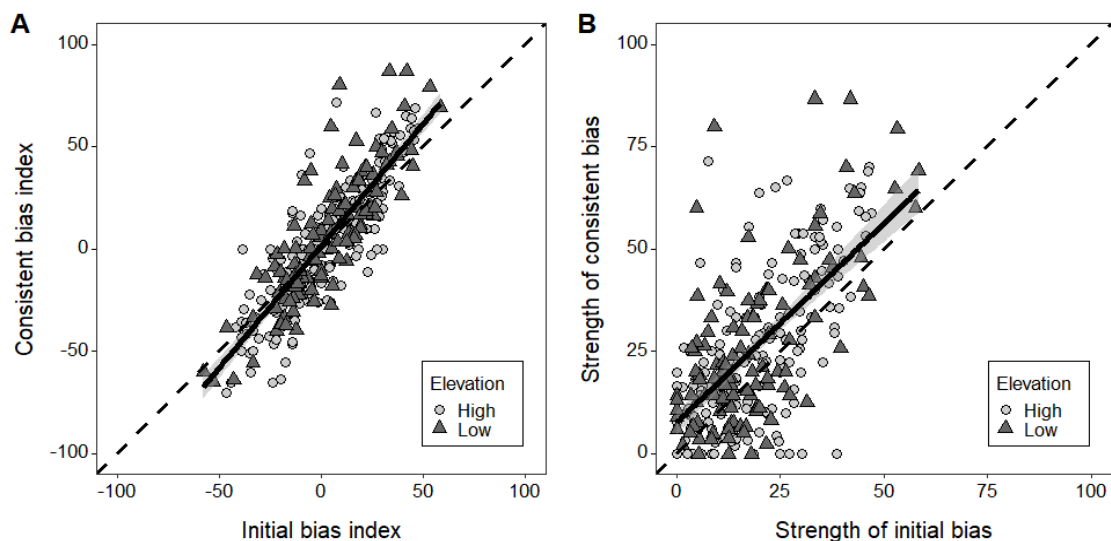


Figure 3.6. Initial versus consistent bias (A) index and (B) strength. All bias estimates use all trials with at least two location errors. Black dashed line indicates a 1:1 relationship. Dark black line indicates linear regression line with 95% confidence interval (shaded areas). For (A), negative index values represent right bias and positive index values represent left bias. For (B), values range from weak or no directional bias (strength = 0) to fully left- or right-biased (strength = 100). N = 294

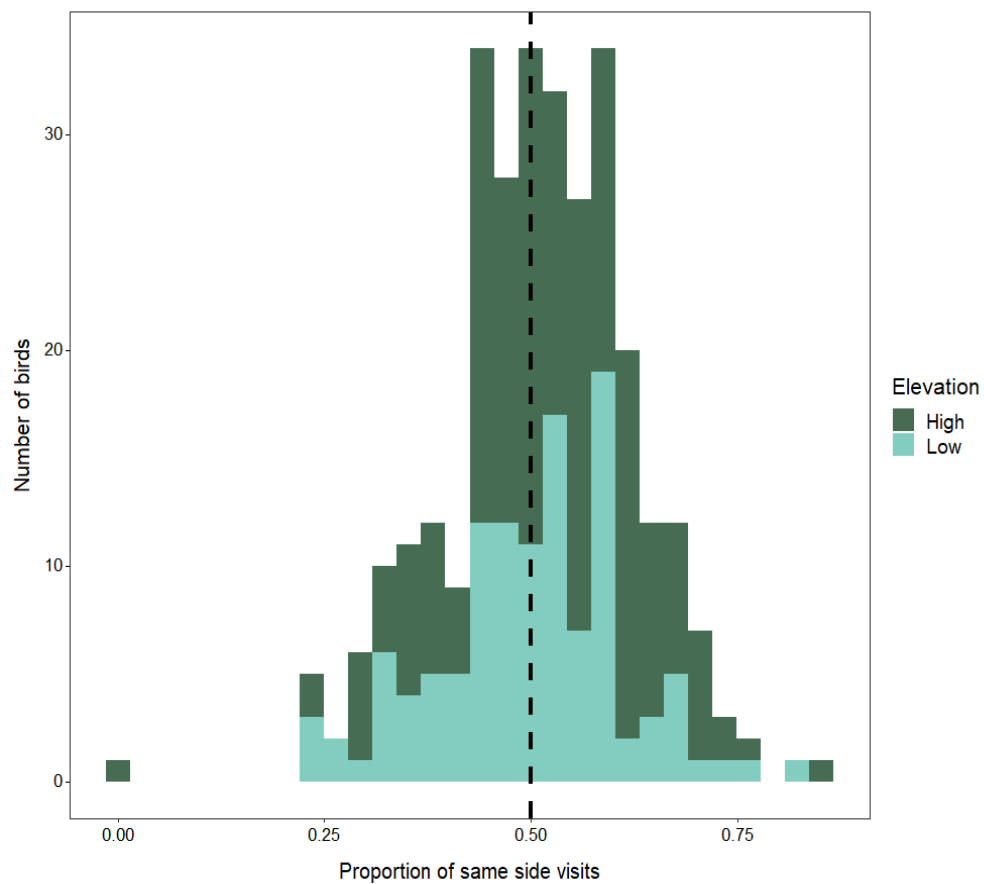
Figure 3.7

Figure 3.7. Proportion of visits to a feeder on the same side of the feeder array as the previous feeder out of the total trials analyzed for initial directional bias. Shown by elevation. Dashed line indicates an equal proportion (0.50). N = 303

Appendix

Table A3.1 Number of birds excluded during data preparation for 2020-2021 and 2019-2020 in order to create initial and consistent bias datasets

Reason for exclusion	Number of birds			
	2020-2021		2019-2020	
	Initial bias	Consistent bias	Initial bias	Consistent bias
Location errors < 2	5	5	4	4
Distance between feeders > 2	1	3	2	3
Trials scored left or right < 6	11	18	9	44
Outlier	1	1	0	0
Final dataset	303	294	206	170

Chapter 4 Learning Predictably Changing Spatial Patterns Across Days in a Food-Caching Bird

L.M. Benedict ^{1*}, V.K. Heinen ¹, B.R. Sonnenberg ¹, E.S. Bridge ², V.V. Pravosudov ¹

¹ Department of Biology, University of Nevada Reno, NV, U.S.A.; ² University of Oklahoma, Oklahoma Biological Survey, Norman, OK, U.S.A.

*Corresponding author, Lauren Benedict Nguyen, published as L.M. Benedict

Publication Citation

Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Bridge, E. S., & Pravosudov, V. V. (2023). Learning predictably changing spatial patterns across days in a food-caching bird. *Animal Behaviour*, 196, 55–81. [doi: 10.1016/j.anbehav.2022.11.005](https://doi.org/10.1016/j.anbehav.2022.11.005)

Abstract

Environmental variability favours the evolution of learning and memory, influencing not only basic associative learning processes but also more advanced cognitive abilities associated with cognitive flexibility. When environmental conditions change repeatedly and predictably, the ability to learn related patterns and anticipate future changes can be highly adaptive. We tested whether food-caching mountain chickadees, *Poecile gambeli*, from different elevations could (1) successfully learn daily alternating food locations in an eight-position spatial serial reversal task across multiple days and (2) use the daily alternating rule to predict the next day's food location under natural conditions. Chickadees learned the alternating, serial reversal task successfully but birds from high elevations with harsher, less predictable winter environmental conditions performed worse than chickadees from milder, more predictable low elevations. In addition, we found evidence that some birds at low but not high elevation were capable of learning to predict which feeder would provide food the next day after switching food locations just seven times. This behavior suggests that chickadees recalled which feeder was rewarding the previous day in order to anticipate the feeder that would provide a food reward on the current day. Overall, our study suggests that food-caching chickadees are highly cognitively flexible and show performance consistent with learning the reversal rule and are able to predict a learned switching pattern, but such flexibility may be a trade-off with stronger spatial memories and higher memory load favored by harsher winter environmental conditions.

Highlights

- Chickadees in the wild showed rule-based learning on a serial reversal task.
- Birds showed environment-based differences in learning a serial reversal task.
- Birds were capable of learning to predict feeder location across days.
- Differences in serial reversal learning are consistent with proactive interference.

Introduction

In variable environments, learning and memory can be highly adaptive, allowing animals to adjust their behavior to varying conditions based on previous experiences (Aoki & Feldman, 2014; Dridi & Lehmann, 2016; Dunlap & Stephens, 2016; Stephens, 1991). Learning is most effective when environments are uncertain (i.e., environmental conditions such as precipitation, temperature and food availability change frequently) and previous experience is reliable (i.e., when learned information leads to the best action in future situations; Dunlap & Stephens, 2016; Stephens, 1991). In these environments, using associative learning to remember relationships between stimuli and responses may provide an advantage (Dunlap & Stephens, 2016). Moreover, if environmental variation repeats nonrandomly, then individuals may be able to learn general rules and even learn how to use these rules to predict (or anticipate) future conditions (e.g., Murphy et al., 2008). This rule-learning approach may be more efficient than repeatedly learning and relearning individual associations, as the latter may interfere with learning new information or with retrieving previous memories through proactive or retroactive interference (Shettleworth, 2010; Tello-Ramos et al., 2019).

Regular environmental changes can be associated with multimodal cues, consisting of visual, auditory, spatial or even temporal stimuli. Basic associative learning and memory abilities can be used to make associations with each environmental cue, but flexibly relearning information when conditions change may be difficult due to proactive interference (Anderson & Neely, 1996; Jacoby et al., 2001; Wixted, 2004). With relearned information, multiple memories may become associated with the same cue and

subsequently “interfere” with each other during memory recall (Bjork, 1989; Tello-Ramos et al., 2018). The strength of proactive interference appears related to the strength of initial memories (i.e., memory longevity and persistence; reviewed in Tello-Ramos et al., 2019) and how similar the cues and contexts are (Lewis, et al., 2013; Rodriguez et al., 1993). As a result, individuals that have better learning and memory ability often perform worse in tests designed to measure flexibility (Bebus et al., 2016; Croston et al., 2017; Hermer et al., 2021; Lewis & Kamil, 2006; Tello-Ramos et al., 2018). This raises questions of how animals may solve complex tasks in natural contexts when conditions change frequently.

The term “behavioral flexibility” has been used to describe a variety of different behaviors (Audet & Lefebvre, 2017), but, traditionally in animal behavior, cognitive flexibility is defined as the ability to rapidly relearn changing associations, allowing animals to better track a changing environment or learn the reversal rules (Badre & Wagner, 2006; Bond et al., 2007; Shettleworth, 2010; Strang & Sherry 2014; Tello-Ramos et al., 2019). Behavioral flexibility is frequently measured in reversal associative learning tasks, either using a single reversal (e.g., Croston et al., 2017) or serial reversals (reviewed in Izquierdo et al., 2017). While both tasks require an animal to learn a reversed contingency, repeatedly reversing the same contingencies during the serial task may introduce different cognitive processes associated with flexibility. A single reversal task requires an individual to inhibit the recall of previously learned associations to associate new information with familiar cues during the reversal (Harlow, 1949; Strang & Sherry, 2014; Tello-Ramos et al., 2019). As there is only one reversed association, individuals can forget the first association to learn the second one. But this strategy poses

a challenge for solving a serial reversal task because after the second reversal, animals need to relearn the first association, despite already learning to completely inhibit that association during the first reversal. Instead, to efficiently solve serial reversal tasks, an animal must learn to retain previous memories of both associations and learn the “switching” or “reversal rule” (Izquierdo et al., 2017).

Learning an abstract rule may integrate different parts of the brain and involve different cognitive processes than basic associative learning, such as executive functioning (Lai et al., 1995). Animals must first inhibit memories of previously learned associations (e.g., unlearning them) and then learn new associations (e.g., Lai et al., 1995). Therefore, one way to measure performance in a serial reversal task is to estimate how well animals learn and remember the reversal locations following each reversal, measured by the number of total errors before making a correct choice (e.g., Croston et al., 2017). Such errors made within a reversal learning context reflect the ability to switch between multiple memories without inhibition, potentially providing major advantages to individuals in variable and predictable environments.

Many changing environmental conditions occur across days, but it is still unclear to what extent animals can use time as a cue in associations or can incorporate time into learning to learn the predictable changes. Although it might be highly adaptive in variable environments to learn and use a rule to predict conditions across days or weeks, the “stuck in time” hypothesis suggests that animals can only respond to stimuli in the present moment and cannot recall specific moments in the past to anticipate future events (Roberts, 2002; Tulving, 1985). Animals are well known to associate a stimulus or

reward with internal cues generated by circadian rhythms, referred to as an individual's "biological clock" (Mistlberger, 2009; Roberts & Feeney, 2009; Suddendorf & Corbalis, 2007). Animals can also anticipate daily rewards (e.g., Biebach et al., 1989), track short time intervals (e.g., Pavlov, 1927; reviewed in Shettleworth, 2010) and remember sequences of events after significant reinforcement (Devine et al., 1979; Roberts, 2002; Shimp, 1976; Shimp & Moffitt, 1974). But less clear are the mechanisms that may underlie the ability to learn to predict changes that occur across multiple days. Without this ability, the benefits of learning a rule may be limited to rules that can be associated with circadian rhythms (i.e., within a ca. 24 h period).

In this study, we designed an experiment to test whether wild food-caching mountain chickadees, *Poecile gambeli*, from two environments differing in environmental harshness and predictability (Croston et al., 2016, 2017; Heinen, Pitera, Sonnenberg, Benedict, Branch et al., 2021; Heinen, Pitera, Sonnenberg, Benedict, Bridge et al., 2021; Pitera et al., 2018; Tello-Ramos et al., 2018) can (1) learn the reversal rule in an eight-position spatial serial reversal learning task with daily location reversals in the wild and (2) learn to predict daily changes in the location of a food reward based on previous experience during the task. Both processes, learning the rules associated with repeated daily changes in a serial reversal task and learning to predict future events, are usually considered "higher-level" cognitive processes, as they involve learning abstract strategies and rules that relate to learned associations (Shettleworth, 2010).

We conducted an eight-position spatial serial reversal task by alternating a rewarding food location each day between one of two locations in an eight-feeder spatial

array (using four previously established feeder arrays; Fig. 4.1). This experimental set-up differed from classic serial reversal tasks in two key ways. First, instead of reversing the feeder locations after a learning criterion or fixed number of trials, rewarding feeder locations were switched every night, so every morning a different feeder provided food until the end of the day. This design allowed us to explore whether individuals could associate the switching rule with a daily change; birds were unlikely to be able to use circadian rhythms to solve this task because food was available at the same time every day (during daylight hours) but at a different location every other day. This design also allowed birds to continue to learn beyond a set learning criterion, but we could still assess performance at the end of each day to confirm that all birds both fully learned the new location and stopped visiting the previous location. Birds could forage *ad libitum*, and daily visits were grouped into “trials”. A trial began when a bird visited a feeder at an array and ended when that bird found the food reward (after which chickadees typically left the array to eat or cache that seed individually; Table 4.1).

The other key difference between our task and a binary choice experiment was that our task presented eight equally likely locations for a food reward. This allowed us to measure the total location errors or the number of nonrewarding feeders that were visited per trial (maximum of 7 total location errors per trial, out of 8 possible feeder locations; Table 4.1). Of these total location errors per trial, we could further differentiate whether birds made a “reversal error” to the previously rewarding feeder during the previous day’s reversal (maximum of one reversal error per trial; Strang & Sherry, 2014; Stanton et al., 2021) or a “neutral error” to another feeder that did not provide a food reward during either reversal (maximum of 6 neutral errors per trial; see Seu et al., 2009 for a

similar four-position single spatial reversal experiment in rats). This design differs from a binary choice reversal task, in which all errors are reversal errors by default, which specifically characterizes an individual's persistence in visiting the reversal location (one way to assess behavioral flexibility, reviewed in Izquierdo et al., 2017). But by differentiating between reversal and neutral errors, we could assess how birds learned the new, reversal association while simultaneously assessing whether birds remembered and relied on memories of the previous reversal location.

Neutral errors in a reversal learning context could provide information about imperfect learning and whether birds use a “win-stay, lose-shift” strategy, or reversal rule, to solve the task. If birds are using this strategy (discussed in Strang & Sherry, 2014 using a binary choice task), then they should only make a single reversal error and no neutral errors during the first trial after each reversal, suggesting that an individual first makes an error to the previously rewarding choice and then immediately switches to the correct choice. When learning a serial reversal task, birds should demonstrate a continuum of learning from full inhibition of memories (i.e., unlearning the previous association) at the beginning of the serial reversal to perfect performance using a “win-stay, lose-shift” strategy towards the end of the task, by fully retaining both memories of rewarded locations. Inhibition of previous memories associated with previous locations being unrewarded can be expected to result in a random search after each reversal, leading to neutral errors immediately following each reversal as an individual searches for the location that was rewarding two days prior. In contrast, completely uninhibited memories of both locations should be associated only with a single reversal error and no neutral errors, consistent with using the reversal rule. Between these two extremes,

animals should go through intermediate stages of incomplete inhibition leading to imperfect memories, associated with moderate neutral errors after each reversal. Accordingly, neutral errors immediately after each reversal measured during the first trial should decrease throughout the task.

While binary choice tasks and other studies rely on reversal errors to measure cognitive flexibility (reviewed in Izquierdo et al., 2017), using neutral errors in addition to reversal errors provides additional detail to understand the resulting effect on learning and performance. Plus, in multiple-choice spatial learning tasks, each spatial location is unique and thus only one reversal error can be made per trial. In this case, using neutral errors provides greater detail to understand whether results are consistent with the effects of proactive interference and the influence of higher-level cognitive processes involving several brain regions including the hippocampus (e.g., Shah et al., 2018). Although this method does not differentiate between these mechanisms, it should capture the end result through variation in reversal learning. Ultimately, cognitive flexibility reflects how well and how quickly an animal learns the serial reversal task given all available choices, and thus measuring performance in a spatial serial reversal task using all potential errors provides the most ecologically relevant measure of flexibility.

We hypothesized that this task involves three types of learning in the following progression: (1) learning the two reversing feeder locations (i.e., forming associations using spatial learning and memory) and maintaining memories for both locations despite one of them being unrewarded for a long period (full day); (2) learning the reversal rule associated with serial reversal (i.e., learning to switch between two rewarding feeders out

of eight feeders using cognitive flexibility within a spatial context without making any neutral errors); and (3) learning to predict the next day's rewarding feeder location (i.e., learning to associate the serial reversal rule with time (i.e., days), so that individuals visit the new, correct location without first checking the previously rewarded location). We use the term "performance" to describe the number of total location errors including both reversal and neutral errors birds made in different contexts. Good performance is reflected by a faster reduction in the number of location errors throughout the serial reversal task (i.e., days of the task). Optimal serial reversal performance is associated with a single reversal error on the first trial after each reversal, when an individual first visits the previously rewarded feeder and then immediately goes to the correct feeder that was rewarding 2 days prior without making any neutral errors. This behavior could be associated with learning the reversal rule based on flexible memories.

We assessed (1) through performance on a spatial learning and memory task and a single reversal task before the serial reversal experiment began. We assessed (2) based on the number of location errors (both reversal and neutral) birds made during the first trial of each day, as each bird's first visit of the day should reveal where a bird expects to find food and does not represent relearning of the new location. Birds that perfectly learned the reversal rule could be expected to make only one error (reversal error) during each reversal or feeder switch (Strang & Sherry, 2014); they should go first to the previous day's rewarding feeder (i.e., the reversal feeder) but then immediately switch to the correct new location without making additional neutral errors. We additionally assessed reversal learning by testing for a reduction in the total number of reversal errors over the first 20 trials across the serial reversal task. Such a reduction is indicative of decreased

persistence in making reversal errors throughout the task. We assessed (3) using the first trial of each day: if birds learned to predict the next feeder in the reversal sequence across days, they should go to the correct feeder without visiting the reversal feeder during the first visit of each day. If birds learned to predict the next location based on the reversal rule, then they should make no reversal errors, indicating that they go directly to the correct rewarding feeder during the first trial of each day. It is possible that performance measured by neutral errors is confounded by basic spatial learning and memory ability, so we additionally measured performance using only neutral errors without reversal errors. In addition, we directly tested whether spatial learning ability may indeed affect performance in our reversal task when measured either by all errors or just by neutral errors. We included individual performance on a spatial learning and memory task (which we have previously demonstrated to reflect learning ability; Branch et al., 2022; Sonnenberg et al., 2019) in all analyses.

We conducted this serial reversal task with wild mountain chickadees in their natural environment across two montane elevations with substantial differences in winter conditions. Winter conditions at higher elevations (ca. 2400m) are consistently harsher (i.e., lower ambient temperatures, longer duration of snow cover) and less predictable (i.e., more frequent and unpredictable snowfall and more severe winter storms, causing unpredictable interruptions in food availability) compared to lower (ca. 1900m) elevations (Heinen, Pitera, Sonnenberg, Benedict, Bridge et al., 2021; Kozlovsky et al., 2018, 2018; Pitera et al., 2018). Differences in daily foraging routines between low and high elevations during the winter were also consistent specifically with differences in environmental predictability (Pitera et al., 2018). If learning a rule is beneficial in more

predictable environments, then we would expect birds from the milder, more predictable environment at low elevation to learn the alternating pattern better than birds from the harsher, less predictable environment at high elevation. Our predictions are based primarily on the possible advantage of learning a rule in predictable environments; however, there is also a difference in harshness across these elevations. We have previously found significant cognitive, morphological and behavioral differences between birds from each of these environments (Croston et al., 2016, 2017; Freas et al., 2012; Heinen, Pitera, Sonnenberg, Benedict, Bridge et al., 2021; Pitera et al., 2018; Tello-Ramos et al., 2018) and have found selection on spatial learning and memory at the high elevation (Sonnenberg et al., 2019). Because there is stronger selection on food-caching propensity and spatial learning and memory ability at higher elevations compared to lower elevations, we predicted that stronger proactive interference associated with stronger memories and higher memory load due to more food caching would negatively affect high-elevation birds flexibility, reflected in their performance in learning a serial reversal task (Tello-Ramos et al., 2019). Similarly, we predicted that older birds would learn the reversal rule better than younger birds, according to previous work documenting differences in cognitive flexibility between these groups (Tello-Ramos et al., 2018).

Methods

Study System

The study was conducted in winter 2020–2021 at Sagehen Experimental Forest in the Sierra Nevada (Sagehen Creek Field Station, University of California Berkeley, located 10 km north of Truckee, CA, U.S.A.) as part of our long-term mountain chickadee study (Croston et al., 2016, 2017; Freas et al., 2012; Kozlovsky et al., 2018; Tello-Ramos et al., 2018). During annual banding efforts (2014–2021), we trapped birds at nestboxes or feeders using mist nets and banded them with unique color bands and a passive integrated transponder (PIT) tag with a unique alphanumeric ID (IB Technology, Leicestershire, U.K.). Age at initial capture was determined as “juvenile” (less than 1 year of age) or “adult” (at least 1 year or older) by using multiple plumage characteristics (Meigs et al., 1983; Pyle, 1997), breeding status and nestling banding records if available. Age in years was determined, when possible, from previous banding records (2013–2021).

Animal Welfare Note

To the best of our knowledge, no birds were harmed by the collection of these data and birds were only handled for a few minutes during banding. We detected no negative effects of using PIT-tag tags and color bands during our study. The study was approved by the University of Nevada Reno Institutional Animal Care and Use Committee (Protocol 00818, 00046 and 00603) and was in accordance with California

Department of Fish and Wildlife Permit D-0011776516-4. For banding efforts, we followed U.S. Federal Bird Banding Permit 22878.

Experimental Apparatus

Cognitive tasks in this study were conducted using spatial arrays of “smart” feeders, established at the study system in 2014 and used annually for cognitive testing (Croston et al., 2016, 2017). Each of four arrays (two per elevation, ca. 1.2 km apart) consisted of eight feeders mounted equidistantly to a 122 × 122 cm aluminum frame and raised 3 m above the ground. Each feeder was equipped with radiofrequency identification (RFID) technology to detect PIT-tagged individuals and control access to food within the feeder via a mechanical door (Bridge & Bonter, 2011). The feeders could be set to three different “modes”: (1) “open” mode, when the feeder doors were always open and food inside was clearly visible; (2) “all” mode, when doors were closed until any PIT-tagged chickadee landed on the perch, triggering the door to open; (3) “target” mode, when doors were closed and were programmed to only open for certain PIT-tagged birds. “Target” mode was used during cognitive tests so that birds could be individually “assigned” to a single rewarding feeder at one array. “Open” and “all” modes were used to habituate birds to the feeders. In all three modes, every visit by a PIT-tagged bird was recorded, whether or not the bird obtained a food reward.

Assessing Spatial Learning and Memory and Cognitive Flexibility

Before conducting the serial reversal experiment, birds were habituated to the feeders during “open” and “all” mode (20 November 2020 – 13 January 2021). To assess spatial learning and memory ability, birds were restricted to only one rewarding feeder in an array (“feeder A”) for 5 days (“target” mode, 13–17 January 2021). Birds were assigned to a rewarding feeder pseudorandomly so that no individual was assigned to their most visited feeder during “open” or “all” mode and the new feeder was always on a different side of the square array. In a single spatial reversal task, birds were reassigned to a different rewarding feeder (“feeder D”) on a different side of the same square array for 5 days (“target” mode, 17–20 January 2021). We use letters “A” and “D” to indicate that assigned feeders within a task were not adjacent, but the assigned feeder locations were not the same for every bird. Groups of birds previously assigned to the same feeder were reassigned to separate feeders to minimize social learning (Tello-Ramos et al., 2018).

Chickadees forage for single seeds, leaving the array to consume or cache the seed, so each visit to the rewarding feeder yields one food item. A “trial” began when a bird first visited a feeder at an array and ended when it received a food reward; all feeder locations visited before finding the food reward were “location errors” (Table 4.1). We used the mean number of location errors per trial in the first 20 trials as a metric of spatial learning and memory ability in the first task and of reversal learning ability in the second task (following our previous work: Croston et al., 2017; Heinen, Pitera, Sonnenberg, Benedict, Branch et al., 2021; Heinen, Pitera, Sonnenberg, Benedict, Bridge et al., 2021;

Heinen, Benedict et al., 2021; Sonnenberg et al., 2019; Tello-Ramos et al., 2018). Our previous work showed that mean performance over the first 20 trials provides an ecologically relevant measure associated with differences in fitness.

Spatial Serial Reversal Tasks

We tested chickadee performance in two separate spatial serial reversal tasks in which the rewarding feeder alternated every day between two locations, switching at night when feeders were inactive so that each new day was another reversal. The first spatial serial reversal (Serial R1) immediately followed the spatial learning and memory and single reversal tasks and used the same feeder assignments assigned to individuals in the previous tasks (e.g., feeders A and D, 20–26 January 2021; Fig. 4.2). The second spatial serial reversal (Serial R2) started 16 days after the end of Serial R1 and used new feeder assignments for all birds (e.g., feeders E and H, 10–26 February 2021; Fig. 4.2). Feeders “E” and “H” were not adjacent to each other or to Serial R1 assignments.

We initially intended to conduct only one spatial serial reversal task over a longer interval to allow for more reversals and more time to detect significant trends. However, Serial R1 was interrupted by an unexpected snowstorm that compelled us to alter the operation of the feeders for the safety of the birds (Fig. 4.2). During the storm, feeders were set to the “open” door mode. After the storm, feeders were reset to “all” mode (5–10 February 2021, at low elevation; 8–10 February 2021, at high elevation) and then set to “target” mode to start Serial R2.

The snowstorm interruption allowed us to design Serial R2 to test specifically whether experience with the two alternating locations affected serial reversal performance. During Serial R2, we reassigned each bird to new feeder locations (feeder E and H) to provide all birds with two new feeders to learn regardless of participation in the previous spatial serial reversal task. Thus, we were able to compare performance of (1) the same birds during Serial R1 and R2 and (2) between birds with and without previous experience in Serial R1. Furthermore, we did not repeat the spatial learning and memory task and single reversal task with the new feeder assignments. Instead, we started to alternate daily feeder assignments immediately, so that birds only had 1 day to learn each new feeder assignment before the second serial reversal task began (instead of 5 days, as in the previous cognitive tasks). This allowed us to assess the possible effect of giving birds less experience with the two feeder locations before beginning the serial reversal. Serial R2 then proceeded exactly the same as Serial R1 for the serial reversal but with more reversals (15 days).

Data were only included for each serial reversal if a bird participated in at least seven consecutive days of the task with at least 20 trials per day (see Appendix, Table A4.1, Fig. A4.1). Consecutive days were essential, because if a bird missed a day, it would miss an entire reversal and would experience a different feeder sequence compared to the other birds (e.g., instead of learning “ADADADA” the bird that missed day 3 of Serial R1 might experience “ADDADA”). We used a minimum of 7 days for both serial reversal tasks because Serial R1 only lasted 7 days. We used a minimum of 20 trials, following our previous work (Croston et al., 2017; Heinen, Pitera, Sonnenberg, Benedict, Branch et al., 2021; Heinen, Pitera, Sonnenberg, Benedict, Bridge et al., 2021; Heinen,

Benedict et al., 2021; Sonnenberg et al., 2019; Tello-Ramos et al., 2018;), as the mean performance during the first 20 trials represents a meaningful measure associated with fitness (Sonnenberg et al., 2019). Using 20 trials per day also ensures individuals had minimum sufficient participation to learn the daily feeder location, since we did not use a learning criterion.

Assessing Serial Reversal Performance

In our system, a bird could make a maximum of seven location errors per trial, of which only one could be a reversal error and six could be neutral errors (Table 4.1). In general, when a bird visits any nonrewarding feeder (making either a reversal or neutral error) and does not find food there, it will visit other feeders until it finds the correct location and receives food. Thus, each bird receives one reward in every trial and leaves the feeder array until it begins another trial.

We expected the birds' behavior to differ between the first reversal and subsequent reversals. After the first location switch (e.g., in the single reversal task), a bird should simultaneously learn the new association and inhibit its previously learned association. As it learns, it should be less likely to make a reversal error in each subsequent trial. But when a bird experiences multiple reversals (e.g., in the serial reversal task), it should learn not to inhibit the previously learned association because both associations are necessary to learn the reversal rule. This learning process should be reflected in performance across reversals, during the first trial after each reversal. In our serial reversal task, if the bird has “unlearned” the previous association, it should treat the

previous reversal location the same as the other nonrewarding feeders, making many errors as it searches for the correct feeder. As the bird learns not to inhibit the previous association, the bird should make fewer neutral errors during the first trial per day but may still make a reversal error, suggesting the bird can recall the location of both feeders. A bird is considered to have fully learned the serial reversal rule when it only makes one reversal error and makes no neutral errors in the first trial: the bird should visit the reversal feeder first, then consecutively visit the correct rewarding feeder (e.g., Strang & Sherry, 2014).

Metrics for Learning to Reverse

Location errors in trials 1–5 to measure performance.

For both serial reversal tasks, we analyzed the number of location errors each bird made per trial in the first five trials per day. We analyzed the first trial separately from trials 2–5 per day because the first trial was entirely based on what birds learned prior to the current reversal. It provides specific information about where the birds expected to find food based on their previous experience when they visited the array for the first time following the night after each reversal (i.e., after being rewarded at a different feeder during the previous day). Performance in trial 1 per day does not represent relearning of the new location during that day. In contrast, during trials 2–5 per day, birds have already found the rewarding feeder during the first trial and thus could use that experience to find the correct feeder. We assessed trials 2–5 per day to test for consistency in performance following trial 1, and we expected to see evidence of learning across these trials because

previous work has found variation in learning within five trials of similar cognitive tasks (Croston et al., 2017). Hereafter, we always refer to the trials “per day” but may refer to these simply as “trial 1” or “trials 2–5”.

Total location errors in the last five trials to assess daily learning.

We also analyzed the sum of location errors in the last five trials per day to assess whether individuals learned the association for that particular day. We used the sum of location errors in the statistical analyses instead of errors per trial to avoid complexities in fitting zero-inflated models, as we expected most trials towards the end of each day to have zero location errors. Performance at the end of each day was important to assess because we did not reverse associations based on individuals reaching a learning criterion, as is more common in the serial reversal literature. If a bird did not learn the feeder location by the end of a given day, we expected it to make more errors than expected by chance (i.e., more than 4.5 errors per trial, or more than 22.5 errors summed across five trials; Tille et al., 1996; assessed using one-sample Wilcoxon signed-rank tests). All birds in our study performed much better than expected by chance and, moreover, showed performance similar what is commonly used as a learning criterion.

We also used the sum of total location errors in the last five trials per day to explore whether birds demonstrated “anticipatory errors” in which individuals make more errors just before the reversal switch. Anticipatory errors have been documented in single “within-session reversal” tasks (i.e., when the reversal occurs once after a fixed number of trials; Rayburn-Reeves et al., 2010; Stagner et al., 2013). In our study, these errors

might suggest birds use timing or counting to switch associations instead of local cues associated with the change in day, and we might see these errors increase throughout the serial reversal task despite improved performance in the first five trials per day.

Proportion of birds with perfect reversal performance.

In addition, we analyzed the proportion of birds that reached perfect serial reversal learning performance (i.e., only making a single error in trial 1, which is a reversal error) out of all the birds that participated each day and how this proportion changed throughout the task.

Metric for Learning to Predict

To assess whether birds learned to predict which feeder would provide food the next day, we tested whether or not birds made a reversal error in trial 1 per day (e.g., Rayburn-Reeves et al., 2011). If a bird learned to predict the alternating locations of the rewarding feeders, it should not make a reversal error in trial 1, instead going directly to the new rewarding feeder. Thus, the probability that a bird makes a reversal error in trial 1 should decrease across reversals (i.e., days) as the bird learns to predict the next feeder. This should coincide with an overall reduction in the number of location errors in trial 1 each day.

Metrics for Assessing Proactive Interference

Previous reversal trials.

To assess the potential association between proactive interference and learning the serial reversal task, we used the total number of trials completed during the previous reversal (“previous reversal trials”) to estimate total experience and potential learning of the reversal feeder location. If proactive interference negatively affects performance in the serial reversal, we expected birds that completed more previous reversal trials (and thus that formed stronger associations with the reversal feeder) would make more location errors. Stronger associations with a location should lead to more inhibition of previous memories and hence more neutral errors would be expected.

Sum of reversal errors in the first 20 trials.

We also analyzed the sum of reversal errors made in the first 20 trials per day (maximum 20 reversal errors; maximum of one per trial) to assess “reversal” persistence, when a bird continued to visit the feeder that was rewarded during the previous day even after it located the new rewarding feeder in trial 1. As birds learn the reversal rule and learn to overcome proactive interference, we should see a reduction in the number of reversal errors across the serial reversal task. If birds at high elevation have more proactive interference than birds at low elevation, we would expect low-elevation birds to reduce the number of reversal errors faster (Croston et al., 2017). For birds that participated in

both serial reversal tasks, we also analyzed the number of reversal errors made in Serial R2 to previously rewarding feeders from Serial R1.

Comparing performance between serial reversal tasks.

We compared performance (location errors in trial 1) between the two serial reversal tasks by (1) comparing birds that participated in both tasks to birds that participated in only one task and (2) comparing performance between tasks within birds that participated in both Serial R1 and R2.

Statistical Analyses

Data from Serial R1 and R2 were analyzed separately due to the different experimental procedures (i.e., the amount of time to learn the two rewarding feeder locations) and possible effects from conducting experiments sequentially. We primarily used linear regression models fitted using R version 4.1.0 (R Core Team, 2018). Before and during model fitting, we checked all assumptions of linear models, using the R package “DHARMA” (Hartig, 2020) to check $Q-Q$ plots, residual by predicted value plots and goodness-of-fit tests. Generalized linear mixed-effects models (GLMM) were fitted for daily data that contained repeated measures (individuals repeated each day). The conclusions of Nakagawa and Cuthill (2007) and Schielzeth et al., (2020) were considered during model fitting and reporting.

Learning the locations.

The two rewarding feeder locations used for Serial R1 were the same as those learned by birds during the 5-day spatial learning and memory task and the 5-day single reversal task (Fig. 4.2). The mean number of location errors per trial over the first 20 trials of each task (not per day) were used to assess performance on each task. These metrics were used as predictor variables to assess learning and memory ability and single reversal ability, respectively, in serial reversal analyses. Separate models were fitted for each cognitive ability to avoid possible covariance, due to the hypothesized trade-off between spatial learning and memory and cognitive flexibility (Tello-Ramos et al., 2018, 2019). The two new locations for Serial R2 were introduced 2 days before the start of Serial R2 (Fig. 4.2), were available for 1 day each and were also evaluated using the mean number of location errors in the first 20 trials. However, we did not use these measures as predictor variables in our models, as these locations were the third and fourth learned locations for birds that had already participated in Serial R1.

For all four feeder locations, we used linear regressions to evaluate how mean performance varied across elevations (high and low) and age groups (juvenile and adult). We used one-sample Wilcoxon signed-rank tests to compare the mean performance for each learned location to chance level (given eight choices, random expectation is 4.5 errors; Tille et al., 1996).

Learning to reverse.

We fitted four Poisson generalized linear mixed-effects models (GLMM) with the number of location errors per bird per day (1) in trial 1, (2) trials 2–5, (3) the sum of the last five trials and (4) in trials 2–5 before making a reversal error. All models included a random intercept of individual bird ID to adjust for repeated measures. All models included the same predictor variables: day of the experiment (i.e., reversal number), elevation (high or low), cognitive task performance (either spatial learning and memory ability from the first 5-day task or single reversal performance from the second 5-day task; Fig. 4.2), either age class (juvenile or adult) or age in years (0–8 years old) and interaction effects of day*elevation and cognitive performance*elevation. We also included the number of previous reversal trials to assess the effect of total experience from the previous reversal, which should be indicative of the strength of the reversal association; more trials can be expected to result in stronger associations. We first fitted models containing all predictor variables and dropped nonsignificant interactions and variables from reported analyses (except for the main day*elevation interaction, which was always included). Numeric variables were centered and scaled, and the previous reversal trials were log-transformed. Models using data from trials 2–5 also included trial number as a categorical variable to compare performance between trials. We did not include trial number as a numeric variable because we did not expect a linear relationship between performance (i.e., learning) and trial number. Models using data from the last five trials per day used the total number of trials completed that day, instead of previous reversal trials, to control for total learning during that reversal. Models using the number

of neutral errors made before making a reversal error during trials 2–5 excluded trials in which birds did not make a reversal error at all.

To assess previous reversal trials in Serial R1, data from the first reversal (day 1) had to be excluded because there were no data from a previous reversal. To assess whether this exclusion biased the data, the models were also fitted using all 7 days of data from Serial R1 without previous reversal trials (Appendix, Table A4.2). There was no difference in the main results.

We also fitted a Gaussian GLMM using the proportion of birds each day that made exactly one reversal error in trial 1, with no neutral errors. The model included a fixed effect of day, elevation and a day*elevation interaction. The model was weighted by the number of total birds per day.

Learning to predict.

We created a binary categorical response variable indicating whether or not a bird made a reversal error in trial 1 per day. We fitted logistic GLMMs using a logit link with a random intercept of individual and fixed effects of day, elevation, age, cognitive ability and the number of previous reversal trials. Learning to predict which feeder would be rewarding each day should be associated with a significantly lower chance of visiting the reversal feeder in trial 1 across the serial reversal task.

Possible effects of proactive interference.

In addition to analyzing the effect of the number of previous reversal trials, we also used GLMMs to compare performance between birds without experience in Serial R1 with birds that participated in both Serial R1 and R2, to assess performance between the two serial reversal tasks and to test for elevational differences in the sum of reversal errors for the first 20 trials across the serial reversal task.

To compare performance of birds that participated in both serial reversal tasks with those that only participated in Serial R2, we fitted a Poisson GLMM model using the number of location errors from trial 1 per day of Serial R2. The model included a random intercept of individual and fixed effects of Serial R1 participation status (categorical), day, elevation, previous reversal trials and interactions of day*elevation and participation*elevation.

To explore differences in performance between the two serial reversal tasks within the same birds, we fitted a Poisson GLMM model using the number of location errors made in trial 1 per day of Serial R1 and R2 using only birds that completed both tasks. The model included a random intercept of individual and fixed effects of day, elevation, previous reversal trials, task (Serial R1 or R2) and interactions of day*elevation and task*elevation.

To assess the sum of reversal errors in the first 20 trials per day of Serial R1 and R2, we fitted three GLMM models with a random intercept of individual and fixed effects of day, elevation, number of previous reversal trials and an interaction of day*elevation. One model used reversal errors made in Serial R1, one model used

reversal errors made in Serial R2, and the third model used errors made in Serial R2 to the feeders assigned during Serial R1. We thus limited the Serial R2 data set to birds that participated in both serial reversal tasks.

Statistical Software

All models were fitted using “lme4” package (Bates et al., 2015) in R version 4.1.0 (R Core Team, 2018) and evaluated using Wald chi-square, and *P* values were calculated from ANOVA tests using the “car” package. Tukey adjusted post hoc pairwise comparisons were calculated using “emmeans” (Lenth, 2020). Residuals were analyzed using “DHARMA” (Hartig, 2020), and modified R^2 values were calculated according to Nakagawa and Schielzeth (2013) using the “performance” package (Ludecke et al., 2020). Seven optimizers were evaluated using “allFit” from the “lme4” package for each model before selecting the “bobyqa” optimizer from the “lme4” package. The number of trials completed on either the previous day or the current day was log-transformed due to a strong right skew. Numeric variables were centered and scaled. Figures were created using “ggplot2” (Wickham, 2016), “ggeffects” (Ludecke, 2018) and “raincloud” plots (Allen et al., 2021).

Ethical Note

To the best of our knowledge, no birds were harmed by the collection of these data and birds were only handled for a few minutes during banding. To band adult and

juvenile birds, we placed up to three plastic color bands around the birds' legs in a process that took no more than 30 s. One of these color bands contained a PIT tag. If the bird had been previously banded with a metal numeric band issued by the U.S. Geological Survey Bird Banding Laboratory, only two color bands were added and the color band containing the PIT tag was placed on the opposite leg as the metal band. We detected no negative effects of using PIT tags and color bands during our study. After bands were attached, birds were visually inspected for age and sex and were measured for wing length using flattened wing length. Total handling time was only a few minutes and birds were released immediately. To reduce stress during handling, banding efforts were not conducted during adverse conditions (i.e., wind, precipitation), birds were banded as soon as possible after capture and total handling time was minimized further during cold temperatures (ca. <5 °C). The study was approved by the University of Nevada Reno Institutional Animal Care and Use Committee (Protocol numbers 00818, 00046 and 00603) and was in accordance with California Department of Fish and Wildlife Permit D-0011776516-4. For banding efforts, we followed U.S. Federal Bird Banding Permit 22878.

Results

We first present results for Serial R1, then for Serial R2, and finally compare both serial reversal tasks to assess evidence for proactive interference. We present results organized by predictions for each serial reversal task: learning the locations, learning to

reverse and learning to predict. Age was not a significant predictor in any GLMM and was removed from reported results (Appendix, Tables A4.3, A4.4, A4.5, A4.6).

Serial R1: Learning the Locations

In the 5-day spatial learning and memory task, birds performed better than chance (one-sample Wilcoxon signed-rank tests: $V = 0$, $P < 0.001$; $\mu = 4.5$ location errors per trial), making an average \pm SE of 1.07 ± 0.38 mean location errors per trial in the first 20 trials. Performance did not vary significantly between elevations or age groups (linear regression: elevation: $F_{1,171} = 0.93$, $P = 0.34$; age: $F_{1,171} = 2.77$, $P = 0.10$; $R^2_{\text{adj}} = 0.01$; $N = 97$).

In the 5-day single reversal task, birds performed better than chance (one-sample Wilcoxon signed-rank tests: $V = 0$, $P < 0.001$; $\mu = 4.5$ location errors per trial), making an average \pm SE of 0.50 ± 0.24 mean location errors per trial in the first 20 trials. Single reversal performance also did not vary significantly between elevations or age groups (linear regression: elevation: $F_{1,171} = 2.56$, $P = 0.11$; age: $F_{1,171} = 0.01$, $P = 0.91$; $R^2_{\text{adj}} = 0.004$; $N = 97$).

Serial R1: Learning to Reverse

Serial R1, trial 1.

During Serial R1, chickadees improved their trial 1 performance, making fewer location errors in trial 1 each subsequent day of the task. Birds at low elevation improved

significantly faster than birds at high elevation ($N = 97$; Table 4.2, Fig. 4.3a). There was no significant effect of the number of previous reversal trials or spatial learning and memory ability on trial 1 performance, but birds with better single reversal ability performed significantly better in trial 1 throughout Serial R1 (Table 4.2).

There were significant interactions between elevation and day and elevation and single reversal ability (Table 4.2, Appendix, Fig. A4.2). Post hoc GLMM analyses indicated that the main effects in the model were driven by birds at low elevation: birds' performance in trial 1 improved significantly with days and birds with better single reversal ability also performed better in trial 1 (Poisson GLMM for low elevation: day: estimate = -0.18, $\chi^2_1 = 15.82$, $P < 0.001$, $N = 46$; single reversal ability: estimate = 0.30, $\chi^2_1 = 30.96$, $P < 0.001$; number of trials previous day: $\chi^2_1 = 0.38$, $P = 0.54$, $R^2_c = 0.27$, $R^2_m = 0.19$; $N = 46$). In contrast, at high elevation, there was no significant effect of day or single reversal ability on performance in trial 1 (Poisson GLMM for high elevation: day: $\chi^2_1 = 0.45$, $P = 0.50$; single reversal ability: $\chi^2_1 = 0.61$, $P = 0.44$; number of trials previous day: $\chi^2_1 = 0.73$, $P = 0.39$; $R^2_c = 0.13$, $R^2_m = 0.01$; $N = 51$).

Birds at both elevations overall performed better than chance in trial 1, on average making only a few location errors by the last day of Serial R1 (mean \pm SE location errors in trial 1 on day 7 at low elevation: 1.33 ± 1.32 ; one-sample Wilcoxon signed-rank test: $V = 577.5$, $N = 46$, $P < 0.001$; mean \pm SE location errors in trial 1 on day 7 at high elevation: 2.25 ± 1.49 ; one-sample Wilcoxon signed-rank test: $V = 2434.5$, $N = 51$, $P < 0.001$).

In Serial R1, the proportion of birds that made one reversal error and no neutral errors in trial 1 was greater at low elevation compared to high elevation and significantly increased at both elevations across days of the task (linear regression: elevation: $F_{1,11} = 7.28$, $P = 0.02$; day: estimate \pm SE = 0.03 ± 0.01 , $F_{1,11} = 12.20$, $P = 0.005$; $R^2_{\text{adj}} = 0.57$; $N = 97$; Fig. 4.4a).

Serial R1, trials 2–5.

After finding the correct feeder in trial 1, chickadees dramatically reduced the number of location errors they made in subsequent trials each day, making an average \pm SE of 0.23 ± 0.64 errors per trial across trials 2–5 in Serial R1 (Fig. 4.3a). In contrast to trial 1, performance on trials 2–5 did not vary significantly across days (Fig. 4.3a) or between elevations. Trial 2–5 location errors were also not significantly associated with spatial learning and memory ability; however, birds that completed more previous reversal trials performed better during trials 2–5 (Table 4.2).

There was no overall effect of single reversal ability on trial 2–5 performance in Serial R1, but there was a significant interaction between elevation and single reversal ability (Table 4.2). Post hoc GLMM indicated that, at high elevation, birds with better single reversal ability also performed better during trials 2–5 (Poisson GLMM: day: $\chi^2_1 = 0.83$, $P = 0.36$; single reversal ability: estimate = 0.24, $\chi^2_1 = 11.04$, $P < 0.001$; number of previous reversal trials: $\chi^2_1 = 3.36$, $P = 0.07$; trial number: $\chi^2_3 = 3.48$, $P = 0.32$; $R^2_{\text{c}} = 0.12$, $R^2_{\text{m}} = 0.06$). In contrast, there was no significant effect of single reversal ability on trials 2–5 at low elevation (Poisson GLMM: day: $\chi^2_1 = 0.002$, $P = 0.97$; single reversal

ability: $\chi^2_1 = 1.67$, $P = 0.20$; number of previous reversal trials: $\chi^2_1 = 1.63$, $P = 0.20$; trial number: $\chi^2_3 = 15.60$, $P = 0.001$; $R^2_c = 0.14$, $R^2_m = 0.04$).

Serial R1, last five trials.

The sum of location errors made in the last five trials per day was overall significantly better than chance (one-sample Wilcoxon signed-rank test: $V = 0$, $N = 97$, $P < 0.001$) and did not vary significantly across days (Table 4.3). Birds at low elevation made significantly fewer location errors in the last five trials (average \pm SE = 0.38 ± 0.736 total location errors in last five trials, $N = 46$) than birds at high elevation (0.52 ± 0.77 total location errors in last five trials, $N = 51$) and their overall performance was consistent with better than 90% criterion (< 0.5 errors/5 trials). Furthermore, birds completing more trials on the same day made fewer location errors in the last five trials; thus, birds with more experience with the daily feeder location had better performance (Table 4.3).

Serial R1: Learning to Predict

Birds, on average, were significantly less likely to make a reversal error in trial 1 as Serial R1 progressed (Table 4.4, Fig. 4.3b). Spatial learning and memory ability was not a significant predictor, but birds that completed fewer previous reversal trials and birds with better single reversal ability were less likely to make a reversal error (Table 4.4).

There was a significant interaction between day and elevation, and post hoc GLMM models showed that the likelihood of making a reversal error only decreased significantly at low elevation, but not at high elevation. On the last day of Serial R1, ca. 21% of birds at low elevation did not make a reversal error in trial 1 (Fig. 4.5a). Moreover, when these birds made no reversal errors, they also made zero or one neutral error (Fig. 4.5b), suggesting that when birds did not visit the previous reversal feeder, they went directly to the correct rewarding feeder instead.

There was also a significant interaction between single reversal ability and elevation (Table 4.4), although the model reported a singular fit and thus interpretation may be limited to chickadees in this sample (see Appendix for further details). Post hoc GLMM models indicated that the effect of single reversal ability was also only present at low elevation, and there was no significant effect of the number of previous reversal trials (logistic GLMM: day: estimate = -0.05, $\chi^2_1 = 7.43$, $P = 0.006$; single reversal ability: estimate = 1.13, $\chi^2_1 = 15.66$, $P < 0.001$; previous reversal trials: $\chi^2_1 = 3.39$, $P = 0.07$; $R^2c = NA$, $R^2m = 0.33$). In contrast, at high elevation, the probability of making a reversal error did not change significantly over time and was not significantly associated with single reversal ability, but more previous reversal trials were associated with a higher probability of making a reversal error (logistic GLMM: day: $\chi^2_1 = 0.87$, $P = 0.35$; single reversal ability: $\chi^2_1 = 3.57$, $P = 0.06$; previous reversal trials: estimate = 0.84, $\chi^2_1 = 5.28$, $P = 0.02$; $R^2c = NA$, $R^2m = 0.12$). Both post hoc models were overfitted and R^2c could not be calculated (reported as $R^2c = NA$). However, we had to include individual ID as a random effect due to pseudoreplication issues.

Serial R2: Learning the Locations

Birds had 1 day to learn the first feeder location of Serial R2 (i.e., feeder E) before Serial R2 began. While learning this location, birds performed better than chance in the first 20 trials (one-sample Wilcoxon signed-rank test: $V = 0$, $N = 235$, $P < 0.001$), making an average \pm SE of 0.68 ± 0.31 mean location errors per trial in the first 20 trials. Juveniles overall performed worse than adults, and there was a significant interaction between elevation and age: juveniles at high elevation performed better than juveniles at low elevation, but there was no difference between elevations for adults (linear regression: elevation: $F_{1,231} = 0.72$, $P = 0.40$; age (categorical): $F_{1,231} = 26.60$, $P < 0.001$; age*elevation: $F_{1,231} = 4.81$, $P = 0.03$; $R^2_{\text{adj}} = 0.12$; $N = 235$).

Next, individuals had one day to learn the second feeder location (i.e., feeder H, first reversal) before Serial R2 began. Birds also performed better than chance in the first 20 trials (one-sample Wilcoxon signed-rank test: $V = 0$, $N = 235$, $P < 0.001$), making an average \pm SE of 0.65 ± 0.36 mean location errors per trial in the first 20 trials. While learning the location of feeder H, juveniles overall performed significantly worse than adults, but there was no significant difference in performance between elevations and no interaction effect between elevation and age (linear regression: elevation: $F_{1,231} = 0.002$, $P = 0.96$; age [categorical]: $F_{1,231} = 10.97$, $P = 0.001$; $R^2_{\text{adj}} = 0.04$; $N = 235$).

Serial R2: Learning to Reverse

Serial R2, trial 1.

Performance in trial 1 improved significantly across days in Serial R2 (Table 4.5, Appendix, Fig. A4.6). There was no overall effect of elevation, but there was a significant interaction between day and elevation: birds at low elevation improved their performance at a higher rate and made significantly fewer location errors than birds at high elevation by the end of Serial R2. Birds that completed more previous reversal trials performed significantly worse in trial 1, but there was no effect of spatial learning and memory ability or single reversal ability (Table 4.4).

In Serial R2, the proportion of birds that made one reversal error and no neutral errors in trial 1 was significantly greater at low elevation compared to high elevation and significantly increased at both elevations across days of the task (linear regression: elevation: $F_{1,27} = 18.12$, $P < 0.001$; day: estimate \pm SE = 0.02 ± 0.002 , $F_{1,27} = 84.09$, $P < 0.001$; $R^2_{\text{adj}} = 0.77$; $N = 235$; Fig. 4.4).

Serial R2, trials 2–5.

After locating the rewarding feeder during trial 1 each day of Serial R2, chickadees again showed almost perfect performance in subsequent trials, making an average \pm SE of 0.22 ± 0.73 location errors across trials 2–5 on the last day of Serial R2 (Fig. 4.6a). In contrast to Serial R1, performance improved significantly across days and differed significantly between elevations (Table 4.5). There was a significant interaction between elevation and day: low-elevation birds performed significantly better than high-elevation birds (Table

4.5). Similarly to Serial R1, birds that completed more previous reversal trials performed significantly worse during trials 2–5, while spatial learning and memory ability and single reversal ability were not significant predictors (Table 4.6).

Serial R2, last five trials.

The sum of location errors made in the last five trials per day was significantly better than would be expected by chance (one-sample Wilcoxon signed-rank test: $V = 0$, $N = 235$, $P < 0.001$), but in contrast to Serial R1, it decreased significantly across days and did not vary significantly between elevations (Table 4.3, Appendix, Fig. A4.5). Birds made an average \pm SE of 0.48 ± 0.88 total location errors in the last five trials per day across the Serial R2, which is consistent with better than 90% learning criterion (<0.5 errors/5 trials). Consistent with Serial R1, better performance in the last five trials of Serial R2 was associated with more trials completed in the same day (Table 4.3).

Serial R2: Learning to Predict

Unlike Serial R1, the probability of making a reversal error in trial 1 did not change significantly with days across Serial R2 ($N = 235$; Table 4.4, Fig. 4.6b). Birds at high elevation were significantly less likely to make a reversal error than birds at low elevation ($N = 235$; Table 4.4). Whereas birds with better spatial learning and memory ability were more likely to make a reversal error in trial 1 ($N = 180$; Table 4.4, Appendix, Fig. A4.6), there was no significant effect of single reversal ability ($N = 164$; Table 4.4).

Consistent with Serial R1, birds that completed more previous reversal trials were also more likely to make a reversal error in trial 1 each day of Serial R2.

Exploring Proactive Interference in Serial R1 and R2

Neutral errors before reversal errors in trial 1.

In Serial R1, during trials in which birds visited the feeder that was rewarding before the switch (i.e., made a reversal error), the number of neutral errors birds made before making a reversal error in trial 1 was very low (average \pm SE = 0.04 ± 0.24 errors; $N = 97$) and did not vary across days, between elevations, with spatial learning and memory ability or with single reversal ability (Appendix, Table A4.7, Fig. A4.4). In contrast, during Serial R2, although the number of neutral errors before a reversal error in trial 1 was also very low (average \pm SE = 0.07 ± 0.31 ; $N = 235$), birds made fewer such errors across days (Appendix, Table A4.8). Also, birds at low elevation and birds with better spatial learning and memory ability made significantly fewer neutral errors before making a reversal error compared to birds at high elevation and birds with worse spatial learning and memory (Appendix, Table A4.8, Fig. A4.4). Consistent with Serial R1, there was no significant effect of single reversal ability (Appendix, Table A4.7, A4.8). Trials in which birds did not make a reversal error were excluded from both analyses.

Comparing performance between Serial R1 and R2.

Birds that participated in both serial reversals ($N = 86$) in general made significantly more trial 1 location errors in Serial R2 both compared to birds that did not participate in Serial R1 at all ($N = 33$, Fig. 4.7B, Table 4.6) and compared to their performance in trial 1 of Serial R1 (Fig. 4.7a, Table 4.6).

Sum of reversal errors in the first 20 trials.

In Serial R1, the sum of reversal errors in the first 20 trials per day did not vary between elevations, across days or with the number of previous reversal trials (Fig. 4.8, Table 4.7; $N = 97$). In contrast, in Serial R2, the sum of reversal errors in the first 20 trials decreased significantly with days across the task and was greater for birds that completed more previous reversal trials (Fig. 4.9, Table 4.7; $N = 86$). In this analysis, we included two types of reversal errors made in the same Serial R2 task: reversal errors made to Serial R1 feeder locations and reversal errors made to the reversal feeder location in Serial R2. But there was not a difference in error type on the sum of reversal errors (Table 4.7).

In Serial R2, the sum of reversal errors in the first 20 trials was significantly greater at high elevation and decreased significantly across days for both reversal errors made to Serial R2 locations and to Serial R1 locations but only varied significantly with previous reversal trials for Serial R2 reversal errors (Table 4.7).

For reversal errors made in Serial R2 to locations used in Serial R1, there was a significant interaction between elevation and day. Post hoc GLMMs suggested that these

reversal errors decreased faster at low elevation and were not affected by the number of previous reversal trials (Fig. 4.9; Poisson GLMM: days: estimate \pm SE = -0.48 ± 0.04 , $\chi^2_1 = 138.95$, $P < 0.001$; number of previous reversal trials: estimate \pm SE = 0.08 ± 0.09 , $\chi^2_1 = 0.82$, $P = 0.36$; $R^2_c = 0.45$, $R^2_m = 0.24$; $N = 40$), compared to high elevation, where the number of previous reversal trials was positively correlated with reversal errors to Serial R1 locations (Poisson GLMM: days: estimate \pm SE = -0.30 ± 0.04 , $\chi^2_1 = 67.61$, $P < 0.001$; previous reversal trials: estimate \pm SE = 0.19 ± 0.09 , $\chi^2_1 = 3.86$, $P = 0.049$; $R^2_c = 0.40$, $R^2_m = 0.11$; $N = 46$).

For reversal errors made in Serial R2 to Serial R2 reversal feeders, there was also a significant interaction between elevation and day. Post hoc GLMMs indicated that at low elevation, these reversal errors decreased across days and were positively correlated with previous reversal trials (Poisson GLMM: days: estimate \pm SE = -0.14 ± 0.04 , $\chi^2_1 = 14.07$, $P < 0.001$; previous reversal trials: estimate \pm SE = 0.25 ± 0.07 , $\chi^2_1 = 11.20$, $P < 0.001$; $R^2_c = 0.06$, $R^2_m = 0.05$; $N = 40$), but there was no significant decrease in the number of these errors at high elevation (Poisson GLMM: days: estimate \pm SE = -0.02 ± 0.03 , $\chi^2_1 = 0.28$, $P = 0.60$; number of previous reversal trials: estimate \pm SE = 0.02 ± 0.06 , $\chi^2_1 = 0.08$, $P = 0.77$; $N = 46$). However, the high-elevation post hoc model had a singular fit, likely due to low variance among birds used as the random intercept ($\sigma^2 < 0.001$).

Supplemental Analyses of Neutral Errors

We also include results of analyses using only neutral errors instead of total location errors to evaluate performance in trial 1 and trials 2–5 for both Serial R1 and R2 (see Appendix, Table A7, Table A8). These results are consistent with our main conclusion and do not suggest that neutral errors were correlated with spatial learning and memory performance.

Discussion

The two main aims of the study were to test whether chickadees in the wild could (1) learn an eight-position spatial serial reversal learning task with daily location reversals by reaching performance consistent with learning the reversal rule and (2) learn to predict the next daily food location based on experience during the serial reversal task. Performance of birds at both elevations was consistent with learning the reversal rule and low-elevation birds consistently outperformed high-elevation birds. Chickadees learned to reverse associations in the two serial reversal tasks, making fewer and fewer location errors in trial 1 each day and making almost no location errors (including no neutral errors) after trial 2 each day. After just seven reversals in Serial R1 and 15 reversals in Serial R2, average performance in trial 1 after the reversal was close to a mean of 1.5 location errors at low elevation and a mean of 2.25 location errors at high elevation. Moreover, the proportion of birds showing trial 1 performance consistent with learning the reversal rule (a single reversal error and no neutral errors, associated with “win-stay; lose-shift”) increased at both elevations by the end of both serial reversal tasks. Again,

this proportion was greater at low elevation than at high elevation. At the same time, birds clearly learned the rewarding feeder location by the end of every day in both tasks, as indicated by fewer than 0.5 mean location errors per trial during the last five trials of the day. This is significantly better than expected by chance and corresponds to better than a 90% learning criterion. Together, these results show that many birds learned to first visit the reversal feeder and then immediately sought out the correct rewarding feeder in the first trial after each reversal, which is consistent with learning the serial reversal rule (Chittka, 1998; Strang & Sherry, 2014).

Improvement in serial reversal performance was mainly due to a reduction in neutral errors during the first trial after each daily switch. In both tasks, birds reduced the total number of location errors in trial 1 across days. Considering that birds could make a maximum of one reversal error per trial, this suggests that birds made fewer neutral errors as they learned the task. In other words, they sampled fewer nonrewarding feeders while searching for the correct rewarding feeder each day across the task. Neutral errors during reversal learning show that birds did not fully retain memories of the previously rewarding feeder following a full day of reinforcement at a different feeder. Moreover, when birds made a reversal error in the first trial each day, they rarely made any neutral errors beforehand. This means that birds typically remembered the most recently visited feeder, and that neutral errors estimated how well they learned to remember the least recent (correct) feeder location. As birds made fewer neutral errors, they learned to remember both feeder locations and flexibly shift between those memories without inhibiting them. This is consistent with the increase in the proportion of birds showing “one-trial, one-reversal-error” performance by the end of both serial reversal tasks. These

results also appear consistent with cognitive flexibility, which is associated with executive function (e.g., Lai et al., 1995) and may allow birds to quickly switch learning contingencies while retaining previous memories.

An alternative explanation for the reduction in neutral errors is that neutral errors could reflect spatial learning and memory ability rather than reversal learning ability. However, if that were the case, then we would expect that birds with better spatial learning abilities would make fewer neutral errors throughout the task. To address this alternative, we measured spatial learning and memory ability before the serial reversal task. Our previous work shows that variation in this ability is associated with significant differences in overwinter survival (Sonnenberg et al., 2019) and has a genetic basis (Branch et al., 2022). Yet variation in spatial learning and memory ability was not significantly associated with differences in neutral errors during the serial reversal task, whether tested as total location errors or separately. Additionally, we found elevational differences in serial reversal performance that were opposite from previous results for spatial learning and memory; specifically, our present results show that birds from low elevation outperformed high-elevation birds for serial reversal, yet we have previously shown that high-elevation birds have better spatial learning and memory abilities than birds at low elevation (Croston et al., 2016, 2017; Freas et al., 2012). We also find the alternative explanation unlikely because learning within a reversal paradigm assumes interference from previous memories, and thus makes serial reversal performance distinct from basic learning and memory ability. Flexibility is measured by how fast animals can learn changing associations, and in the case of serial reversal, how fast animals can learn

repeatedly changing learning contingencies. To measure the rate of serial reversal learning is to measure the reduction in all possible errors across serial reversals.

We also found that learning the serial reversal in Serial R1 was not necessarily determined by persistence to the reversal feeder. Although the number of reversal errors that birds made during the first 20 trials per day did not change at either elevation across Serial R1, birds at low elevation still significantly improved performance in trial 1 throughout the task. This shows that such learning can occur independently of changes in reversal errors. Considering that trial 1 was the first trial after the rewarding feeder location switches, at this point birds did not have experience with the new day's rewarding feeder. As such, improved performance in trial 1 suggests that birds were learning the reversal rule, not relearning the associations each day. During Serial R2, overall improvement in serial reversal performance was associated with both a reduction in the number of neutral errors during trial 1 and a reduction in the sum of reversal errors over the first 20 trials, with high-elevation birds showing worse performance in both compared to low-elevation birds. It is not clear why we observed such differences between Serial R1 and Serial R2, but it is likely related to differences in timing to learn the reversing locations (e.g., 5 days versus 1 day) before each task.

We found mixed support for chickadees' ability to learn to predict the next daily food location based on experience during the serial reversal task by the end of Serial R1 at low elevation (ca. 21% of birds demonstrated an ability to predict which feeder would provide food the next day). As Serial R1 progressed, low-elevation birds overall made fewer location errors in trial 1 and were less likely to make a reversal error, instead

making no or few neutral errors in trial 1. Together, these results suggest that these birds both learned the daily feeder switching pattern and were able to predict which feeder would provide food the next day based on their memory of the previously rewarded feeders in the serial reversal. Being able to predict future locations supports the hypothesis that chickadees learned the reversal rule and then used that rule to predict the daily pattern of switching. Considering that these birds were able to learn to predict the reversal pattern in just seven reversals, it is likely that given more time, more birds might be expected to show this behavior. In contrast, there was no evidence that high-elevation birds learned to predict the reversal pattern despite many showing “perfect” performance consistent with learning the reversal rule and only making a single error during trial 1 each day. These results suggest that high-elevation chickadees are overall less cognitively flexible than low-elevation birds.

There was also no evidence that birds were able to learn to predict the correct rewarding feeder at either elevation during Serial R2, potentially due to the increased difficulty of the task. Serial R2 used two new rewarding locations, requiring birds that had already participated in Serial R1 to learn two additional locations. Additionally, in the first task, birds had 5 days to learn the association with each feeder location used in Serial R1, whereas in the second task, birds only had 1 day to learn each location before starting Serial R2. Considering that proactive interference may be stronger with more recent memories (Storm & Bjork, 2016), we think that providing 5 days to learn each association may have increased the retention interval (i.e., time) between learning the first feeder location and beginning to reverse. In this case, the 1-day retention interval in Serial R2 would be shorter than the 5-day retention interval for Serial R2, potentially

leading to stronger proactive interference when the reversals began and making Serial R2 more difficult. Accordingly, across days in Serial R2, birds also improved performance in the last five trials per day and made fewer reversal errors in the first 20 trials each day. These improvements contrast with Serial R1, during which birds at low elevation showed no improvement in the number of reversal errors across days while greatly reducing the number of neutral errors.

To our knowledge, this is the first study to explore a multiple position serial reversal task using spatial reversals associated with changing days under natural conditions. Birds in this study learned both serial reversal tasks quickly in just seven and 15 daily reversals in each task. Birds also reduced location errors dramatically within each reversal: in just the first two to five trials of the first reversal (day 1), birds made less than 0.25 mean location errors per trial in Serial R1 and less than 0.75 mean location errors per trial in Serial R2. Such performance is in stark contrast to many laboratory-based studies showing that other species require many more trials to learn a serial reversal task (Bond et al., 2007; Bublitz et al., 2017; Cauchoix et al., 2017; Hermer et al., 2018, 2021; Liu et al., 2016; Mackintosh & Cauty, 1971; Madden et al., 2018; van Horik et al., 2019) and rarely reach the levels of performance observed in wild chickadees. However, these differences may be attributed to differences in study design: in these previous studies, each reversal switched after the individual reached a learning criterion and individuals only had two choices that were closely situated. These differences in design mean that individuals in these studies completed fewer trials per reversal compared to our study. Plus, the costs of sampling all choices would likely be lower in the binary choice tasks compared to our eight-position task. Serial reversal performance

may also differ in difficulty across spatial and nonspatial contexts, resulting in further differences between our results and the literature. However, we find these explanations unlikely. Completing more trials per reversal, as in our study, could result in stronger associations and higher levels of proactive interference, making our task more difficult than those in the literature. In food-caching chickadees, spatial associations may also be more difficult to learn than cue-based associations (Pravosudov & Clayton, 2001, 2002). Yet wild chickadees in their natural environment showed faster learning compared to previous laboratory studies, suggesting the exceptional performance of chickadees in our study was likely not due to the study design.

Considering that by the end of both reversal tasks, almost half the birds showed “one-trial, one-error” reversal performance, the performance of chickadees on these serial reversal tasks is consistent with learning the reversal rule (e.g., “win-stay, lose-shift”). This rule is based on flexible memories, as an individual must remember both locations in order to first visit the most recently rewarding location and then find the currently rewarding location without making any other errors. The fact that some low-elevation birds learned to predict the future locations also suggests that the preceding “one-trial, one-error” performance involves learning the rule. Learning to predict requires birds to first learn to keep multiple memories of switching locations without inhibiting them after each reversal and then learn the switching rule that can be used to make predictions.

Elevational Differences and Proactive Interference

We predicted that birds from a more predictable and milder winter environment (e.g., low elevation) would be better at learning the reversal rule and predicting the next reversal feeder than birds from a less predictable winter, harsher environment (e.g., high elevation). This prediction was based on previously documented differences in spatial learning and memory ability, memory retention (Freas et al., 2012) and memory load associated with differences in food caching (Freas et al., 2012). We found support for our prediction: (1) birds at low elevation consistently outperformed birds at high elevation during trial 1 of both serial reversal tasks, (2) a greater proportion of birds learned the reversal task faster at low elevation, (3) only birds at low elevation showed an ability to learn to predict the next rewarding feeder during Serial R1 and (4) birds at low elevation made fewer reversal errors during the first 20 trials during Serial R2. We have previously shown that birds at low elevation are more cognitively flexible on a single spatial reversal task compared to birds at high elevation during some years (Croston et al., 2017; Tello-Ramos et al., 2018). These data suggest that cognitive flexibility is associated with learning to predict changing locations across days. However, we did not detect differences between elevations in single reversal ability or learning and memory ability during this study. This may suggest that the differences between elevations in learning to reverse and predict could be due to other memory related-cognitive differences. This explanation is supported by previous work showing that birds at high elevation in our system are under strong natural selection for spatial learning and memory ability but not for spatial reversal learning ability and typically show better learning and memory performance than birds at low elevation (Croston et al., 2016, 2017; Freas et al., 2012;

Sonnenberg et al., 2019). Alternatively, these elevational differences could be driven by differences in caching and the associated memory load (Tello-Ramos et al., 2019). Birds at high elevation typically cache more than birds at low elevation (Freas et al., 2012); therefore, these birds may have had higher memory load and longer memory retention (Freas et al., 2012), which could interfere more with learning a serial reversal task (Tello-Ramos et al., 2019). In addition, Tello-Ramos et al., (2019) suggested that food-caching species do not need to retain memories associated with retrieved caches; instead, they likely simply forget these locations, eliminating the need to keep updating their memory of retrieved caches. Such forgetting seems to be associated with hippocampal neurogenesis rates, which are higher in birds at high elevation (Freas et al., 2012; Tello-Ramos et al., 2019). Overall, elevational differences in performance appear to be consistent with differences in proactive interference associated with previous memories negatively affecting repeatedly learning and recalling new information.

Assessing Support for Proactive Interference

We think several key results suggest that proactive interference may affect performance and explain differences in performance between serial reversal tasks and elevations. First, birds that completed more trials in the previous reversal during Serial R2 (1) made more location errors, (2) were more likely to make a reversal error in trial 1 each day and (3) made more reversal errors in the first 20 trials per day. This suggests that more experience learning the reversal association was correlated with both worse performance while learning the new association in the subsequent reversal and more

persistence in visiting the reversal feeder. This is exactly in line with predictions of proactive interference (Tello-Ramos et al., 2019). Furthermore, our results are consistent with higher levels of proactive interference at high elevation than at low elevation: in Serial R1 we only see the association between the number of previous reversal trials and making a reversal error in trial 1 at high elevation. In contrast, some birds at low elevation, but none at high elevation, were also able to predict the next reversal location and may have demonstrated higher cognitive flexibility.

Furthermore, performance in Serial R2 was overall worse than in Serial R1. One explanation for this may be the difference in time to learn the first reversal: 1 day in Serial R2 compared to 5 days in Serial R1. More time to learn the single reversal may have allowed individuals in Serial R1 to make an association with each rewarding location but also have more time to unlearn or inhibit the recall of the reversal feeder location. Both stronger memories and more time to overcome the initial interference may have helped birds reform the associations between cues and memories in order to learn the reversal rule.

Another alternative explanation for these differences between reversal tasks could be that birds responded to the snowstorm that interrupted Serial R1 and R2. If this storm increased birds' perception of environmental harshness or unpredictability, birds may have relied more on previous memories to find the rewarding feeder. Or, under this paradigm, it may be more adaptive under harsh or changing conditions to sample previously rewarding feeders, leading to worse performance during Serial R2. However, our previous work shows that chickadees will reduce, rather than increase, sampling of

food sources that have stopped rewarding (Benedict et al., 2021). Furthermore, we find the snowstorm explanation less likely because we would not expect cognitive traits to vary widely with immediate environmental conditions. We also would have then expected to see a more dramatic decrease in performance at high elevation, where conditions were more affected by the snowstorm.

Birds without any Serial R1 experience performed better in trial 1 per day of Serial R2 compared to birds that participated in both serial reversal tasks, which is also consistent with a detrimental effect of proactive interference on learning the second serial reversal task. These differences also appear in line with proactive interference and memory load limitations (Hermer et al., 2021; Tello-Ramos et al., 2019), as birds that learned both tasks needed to learn more feeder locations than birds that only learned one task. We have previously shown evidence of proactive interference in chickadees at the population level using a single reversal task (Croston et al., 2017; Tello-Ramos et al., 2018), and a similar effect of proactive interference on reversal performance was shown in captive great tits, *Parus major*, on an individual level (Hermer et al., 2021).

In further support of the proactive interference hypothesis, our data on the sum of reversal errors in the first 20 trials per day provides an estimate of how birds persisted in visiting the reversal feeder when learning each reversal. For birds that participated in both serial reversal tasks, we measured Serial R2 reversal errors during the first 20 trials to both the reversal location in Serial R2 and to the two rewarding locations used in Serial R1. We found that low-elevation birds in Serial R2 made fewer reversal errors of both types across days, but high-elevation birds only reduced the number of reversal errors

associated with Serial R1 and continued to make reversal errors to the reversal feeder in Serial R2. Overall, birds at high elevation made significantly more reversal errors of both types compared to birds at low elevation, which again is consistent with our hypothesis of elevation-related differences in proactive interference (Croston et al., 2017; Tello-Ramos et al., 2018, 2019).

A possible alternative to the proactive interference hypothesis is that birds made more reversal errors because persistence to visit previously rewarding feeders could be advantageous. Such persistence could be beneficial in environments with frequently or unpredictably replenishing resources, leading individuals to forage from familiar locations instead of using energy to explore new resources. A key aspect of this “adaptive persistence” hypothesis is the idea that making reversal errors reflects an adaptive behavior rather than a by-product of proactive interference. However, within this framework, we would expect chickadees to persist at unrewarding feeders even after those feeders stop providing food, particularly at high elevation where conditions are harsher. Yet, a previous study found the opposite: chickadees at both elevations significantly reduced visits to the previously rewarding feeders after the feeders stopped providing food, with no differences between elevations (Benedict et al., 2021). As such, further evidence is necessary to explore persistence in other contexts and study systems.

Overall, our results show that low-elevation birds are more cognitively flexible, as they were faster and better at learning the serial reversal task and at learning to predict the switching location based on a daily changing pattern, regardless of potential differences in proactive interference levels between elevations. We also show that at least some

differences in serial reversal performance between elevations are consistent with differences in proactive interference levels. The only unexplained and contradictory result was that high-elevation birds were less likely to make a reversal error during trial 1 of the Serial R2 compared to birds from low elevation. But this difference was reversed in trials 2–5, which, combined with all other evidence presented above, strongly suggests that the overall levels of proactive interference were higher at high elevation.

Age Not Correlated with Serial Reversal Performance

We did not find support for our prediction that adults would be more cognitively flexible compared to juveniles and would perform better on the serial reversal task. There were no significant associations between age and serial reversal performance or learning to predict. We also did not find a difference between age groups in single reversal ability (assessed before Serial R1); however, adults performed significantly better than juveniles while learning the locations for Serial R2. While this latter result is consistent with our previous results, suggesting that juveniles were slower to learn a single reversal than adults (Tello-Ramos et al., 2018), we are unsure why we did not see this effect on the single reversal task before Serial R1. On the other hand, chickadees of all ages (up to 8 years old) showed similar performance on the serial reversal, which is consistent with our previous work indicating that chickadees do not show age-related cognitive senescence up to 8 years of age (Heinen, Pitera, Sonnenberg, Benedict, Branch et al., 2021).

No Evidence of Anticipatory Errors

We found that birds made significantly fewer location errors than expected by chance during the last five trials per day of both Serial R1 and R2, suggesting that birds learned the feeder location by the end of their participation each day. This was important to confirm because we did not use a learning criterion to cue each reversal. But regardless, chickadees made, on average, less than 0.5 errors in the last five trials, which is consistent with better than 90% success. We also found that the number of location errors either did not change (Serial R1) or decreased (Serial R2) across days, providing evidence against anticipatory errors. As such, we do not believe birds associated the reversal switch with counting or timed intervals.

Learning to Predict and Implications for Mental Time Travel

The “stuck-in-time” hypothesis argues that nonhuman animals cannot remember events associated with a specific point in time (i.e., episodic memory) or anticipate events far out in the future (Roberts, 2002; Roberts & Feeney, 2009). Yet, in our study, some birds at low elevation demonstrated an ability to predict a spatial location based on their previous experience within just seven reversals in 7 days. Although many birds continued to visit the previous feeder first in trial 1, apparently relying on memory instead of learning to predict, approximately 21% of birds at low elevation in Serial R1 went directly to the correct feeder during their first trial without making a reversal error. While these birds first learned the reversal rule to switch feeders, our results suggest they may have also associated this rule with time across days in order to predict the next feeder

location. Such an abstract association may involve a higher-level cognitive process to integrate memories of where they found food the previous day, where they found food 2 days prior and that the food location alternated between these two locations each day. Each day, a bird should remember which feeder was rewarded the previous day to make a correct prediction about the location of the currently rewarding feeder. Although our study design did not provide evidence for a specific mechanism, our data show that these birds had to associate previous experiences with several points in time to correctly predict where the feeder would be and decide which feeder to visit first the next day. Even though our study was conducted with wild birds in their natural environment without strict control groups, it suggests that chickadees may not be “stuck in time.”

We think our results suggest that a food-caching bird may have used mental time travel (MTT) to associate the reversal rule with days. MTT is a cognitive process in which individuals use semantic memory (knowledge of facts) and episodic memory (remembering past experiences) to reconstruct memories of past events that occurred at a particular point in time (Roberts, 2002). MTT can be used to represent future events that have not occurred yet, allowing individuals to flexibly project their past experiences to simulate potential outcomes of future events (Suddendorf & Corbalis, 2007). While it is extremely difficult to study MTT in nonhuman animals, especially in the field, we think our study design allowed us to investigate several abilities that would be required for chickadees to be able to use MTT. Previous work has shown that black-capped chickadees, *Poecile atricapillus*, are capable of the episodic-like memory and can use semantic knowledge from previous experience to predict future needs and plan accordingly (Feeney et al., 2011). Our study builds on this work, suggesting that

mountain chickadees can (1) associate two feeder locations with two different points in time and (2) can use that information to decide their future action outside of a 24 h period. Both these behaviors have been associated with mental time travel (Cheke & Clayton, 2010; Roberts & Feeney, 2009; Suddendorf & Corbalis, 2007).

There are potential alternative explanations for our results, such as using simple rules to track time. For example, birds may choose to visit the feeder that is least recent in their memory (e.g., Roberts et al., 2008), even though it is unclear what cue they might use to switch locations each day. Or birds may learn that the feeder location switches after roughly a day, but without associating this change with their specific past experience of foraging at the feeder (Cheke & Clayton, 2010). Another possibility is that the birds may be using a simpler rule to predict the next location each day (e.g., counting time between their last visit per day and their first visit the next day), but we find this unlikely because there was variation in how frequently birds visited the feeders each day. Specifically, time intervals between trials ranged from seconds to hours and different birds visited the feeders at different times of day, so it is unlikely that all birds were associating the switching food reward with the same time cue (i.e., dawn). We also did not observe any anticipatory errors to the next rewarding feeder in the last five trials of each day, which would be likely if birds had associated the switching feeder with counting or an imprecise circadian rhythm (e.g., Rayburn-Reeves et al., 2011).

Conclusions

Overall, our data generally supported our main predictions that (1) chickadees are highly cognitively flexible and can learn a serial reversal task across days under natural conditions, reaching perfect “one-trial, one-error” performance consistent with learning the reversal rule; (2) chickadees from low elevation with a milder and more predictable environment would learn the reversal task and reach the perfect “one-trial, one-error” performance significantly faster compared to birds from a harsher and more unpredictable high elevation and (3) chickadees are capable of learning to predict a rewarding feeder after learning the reversal rule across multiple days, albeit only at low elevation. Our data are largely consistent with the effects of proactive interference on serial reversal learning, in which strong spatial memories and the strength of learned associations may negatively affect cognitive flexibility. Furthermore, the ability to learn to predict a spatial location based on previous experience may involve cognitive flexibility and may suggest that these birds are able to associate a rule with more abstract concepts, such as time.

Acknowledgements

This work was supported by the U.S. National Science Foundation (NSF IOS1856181 and IOS2119824 to V.V.P.). L.M.B. and B.R.S. were supported by the NSF Graduate Research Fellowship Program. Thank you to the staff of Sagehen Experimental Forest and Sagehen Creek Field Station (University of California Berkeley) for assistance. We also thank Carrie L. Branch and Angela M. Pitera for feedback during the

writing process. Constructive comments from two referees significantly improved the manuscript. We particularly appreciate thought-provoking and constructive comments from Dr Arnon Lotem, which forced us to think critically about our data and interpretation of our results.

Data Availability

Data will be made available on request.

CRedit Author Statement

Lauren M. Benedict: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original draft preparation, Writing – Reviewing and editing, Visualization.

Virginia K. Heinen: Methodology, Investigation, Data curation, Writing – Reviewing & editing. **Benjamin R. Sonnenberg:** Investigation, Writing – Reviewing & editing. **Eli S.**

Bridge: Methodology, Writing- Review & editing. **Vladimir V. Pravosudov:** Conceptualization, Methodology, Investigation, Writing – Original draft preparation, Writing – Reviewing and editing, Funding acquisition, Project administration, Supervision.

References

- Allen, M., Poggiali, D., Whitaker, K., Rhys Marshall, T., & Kievit, R.A. (2021). Raincloud plots: A multi-platform tool for robust data visualization [version 2; peer review: 2 approved]. *Wellcome Open Research*, 4, 63. <https://doi.org/10.12688/wellcomeopenres.15191.2>
- Anderson, M.C., & Neely, J.H. (1996). Interference and inhibition in memory retrieval. In E. L. Bjork & R. A. Bjork (Eds.), *Memory*, (pp. 237–313). Academic Press. <https://doi.org/10.1016/B978-012102570-0/50010-0>
- Aoki, K., & Feldman, M.W. (2014). Evolution of learning strategies in temporally and spatially variable environments: A review of theory. *Theoretical Population Biology*, 91, 3–19.
- Audet, J., & Lefebvre, L. (2017). What's flexible in behavioral flexibility? *Behavioral Ecology*, 28, 943–947. <https://doi.org/10.1093/beheco/ax007>
- Badre, D., & Wagner, A.D. (2006). Computational and neurobiological mechanisms underlying cognitive flexibility. *Proceedings of the National Academy of Sciences of the United States of America*, 103(18), 7186–7191. <https://doi.org/10.1073/pnas.0509550103>
- Barr, D.J., Levy, R., Scheepers, C., & Tily, H.J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, 68(3), 255–278. <https://doi.org/10.1016/j.jml.2012.11.001>
- Bates, D., Machler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bebus, S.E., Small, T.W., Jones, B.C., Elderbrock, E.K., & Schoech, S.J. (2016). Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. *Animal Behaviour*, 111, 251–260.
- Biebach, H., Gordijn, M., & Krebs, J.R. (1989). Time-and-place learning by garden warblers, *Sylvia borin*. *Animal Behaviour*, 37, 353–360.
- Bond, A.B., Kamil, A.C., & Balda, R.P. (2007). Serial reversal learning and the evolution of behavioral flexibility in three species of North American corvids (*Gymnorhinus cyanocephalus*, *Nucifraga columbiana*, *Aphelocoma californica*). *Journal of Comparative Psychology*, 121(4), 372–379. <https://doi.org/10.1037/0735-7036.121.4.372>
- Branch, C.L., Semenov, G.A., Wagner, D.N., Sonnenberg, B.R., Pitera, A.M., Bridge, E.S., Taylor, S.A., & Pravosudov, V.V. (2022). The genetic basis of spatial

- cognitive variation in a food-caching bird. *Current Biology*, 32, 210–219.
<https://doi.org/10.1016/j.cub.2021.10.036>
- Bridge, E.S., & Bonter, D.N. (2011). A low-cost radio frequency identification device for ornithological research. *Journal of Field Ornithology*, 82(1), 52–59.
<https://doi.org/10.1111/j.1557-9263.2010.00307.x>
- Bublitz, A., Weinhold, S.R., Strobel, S., Dehnhardt, G., & Hanke, F.D. (2017). Reconsideration of serial visual reversal learning in octopus (*Octopus vulgaris*) from a methodological perspective. *Frontiers in Physiology*, 8, Article 54.
<https://doi.org/10.3389/fphys.2017.00054>
- Cauchoix, M., Hermer, E., Chaine, A.S., & Morand-Ferron, J. (2017). Cognition in the field: Comparison of reversal learning performance in captive and wild passerines. *Scientific Reports*, 7(1), Article 12945.
<https://doi.org/10.1038/s41598-017-13179-5>
- Cheke, L.G., & Clayton, N.S. (2010). Mental time travel in animals. *Wiley Interdisciplinary Review of Cognitive Science*, 1, 915–930.
<https://doi.org/10.1002/wcs.59>
- Chittka, L. (1998). Sensorimotor learning in bumblebees: Long-term retention and reversal training. *Journal of Experimental Biology*, 201, 515–524.
<https://doi.org/10.1242/jeb.201.4.515>
- Croston, R., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., Bridge, E.S., Parchman, T., & Pravosudov, V.V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123, 139–149. <https://doi.org/10.1016/j.anbehav.2016.10.004>
- Croston, R., Kozlovsky, D.Y., Branch, C.L., Parchman, T.L., Bridge, E.S., & Pravosudov, V.V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behaviour*, 111, 225–234. <https://doi.org/10.1016/j.anbehav.2015.10.015>
- Devine, J.V., Burke, M.W., & Rohack, J.J. (1979). Stimulus similarity and order as factors in visual short-term memory in nonhuman primates. *Journal of Experimental Psychology: Animal Behavior Processes*, 5, 335–354.
<https://doi.org/10.1037/0097-7403.5.4.335>
- Dridi, S., & Lehmann, L. (2016). Environmental complexity favors the evolution of learning. *Behavioral Ecology*, 27, 842–850.
<https://doi.org/10.1093/beheco/arv184>
- Dunlap, A.S., & Stephens, D.W. (2016). Reliability, uncertainty, and costs in the evolution of animal learning. *Current Opinion in Behavioral Sciences*, 12, 73–79.

- Feeney, M.C., Roberts, W.A., & Sherry, D.F. (2011). Black-capped chickadees (*Poecile atricapillus*) anticipate future outcomes of foraging choices. *Journal of Experimental Psychology: Animal Behavior Processes*, *37*, 30–40. <https://doi.org/10.1037/a0019908>
- Freas, C.A., LaDage, L.D., Roth, T.C., II, & Pravosudov, V.V. (2012). Elevation-related differences in memory and the hippocampus in mountain chickadees, *Poecile gambeli*. *Animal Behaviour*, *84*(1), 121–127. <https://doi.org/10.1016/j.anbehav.2012.04.018>
- Harlow, H.F. (1949). The formation of learning sets. *Psychological Review*, *56*, 51–65.
- Hartig, F. (2020). DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models (Version 0.2.7). <https://CRAN.R-project.org/package=DHARMA>
- Heinen, V.K., Pitera, A.M., Sonnenberg, B.R., Benedict, L.M., Branch, C.L., Bridge, E.S., & Pravosudov, V.V. (2021). Specialized spatial cognition is associated with reduced cognitive senescence in a food-caching bird. *Proceedings of the Royal Society B: Biological Sciences*, *288*, Article 20203180. <https://doi.org/10.1098/rspb.2020.3180>
- Heinen, V.K., Pitera, A.M., Sonnenberg, B.R., Benedict, L.M., Bridge, E.S., Farine, D.R., & Pravosudov, V.V. (2021). Food discovery is associated with different reliance on social learning and lower cognitive flexibility across environments in a food-caching bird. *Proceedings of the Royal Society B: Biological Sciences*, *288*, Article 20202843. <https://doi.org/10.1098/rspb.2020.2843>
- Heinen, V.K., Benedict, L.M., Pitera, A.M., Sonnenberg, B.R., Bridge, E.S., & Pravosudov, V.V. (2021). Social dominance has limited effects on spatial cognition in a wild food-caching bird. *Proceedings of the Royal Society B: Biological Sciences*, *288*, Article 20211784. <https://doi.org/10.1098/rspb.2021.1784>
- Hermer, E., Cauchoix, M., Chaine, A.S., & Morand-Ferron, J. (2018). Elevation-related difference in serial reversal learning ability in a nonscatter hoarding passerine. *Behavioral Ecology*, *29*(4), 840–847. <https://doi.org/10.1093/beheco/ary067>
- Hermer, E., Murphy, B., Chaine, A.S., & Morand-Ferron, J. (2021). Great tits who remember more accurately have difficulty forgetting, but variation is not driven by environmental harshness. *Scientific Reports*, *11*, Article 10083.
- Izquierdo, A., Brigman, J.L., Radke, A.K., Rudebeck, P.H., & Holmes, A. (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience*, *325*, 12–26.

- Jacoby, L.L., Hay, J.F., & Debnar, J.A. (2001). Proactive interference, accessibility bias, and process dissociations: Valid subjective reports of memory. *Journal of Experimental Psychology*, *27*, 686–700.
- Kozlovsky, D.Y., Branch, C.L., Pitera, A.M., & Pravosudov, V.V. (2018). Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *Royal Society Open Science*, *5*, Article 171604. <https://doi.org/10.1098/rsos.171604>
- Lai, Z.C., Moss, M.B., Killiany, R.J., Rosene, D.L., & Herndon, J. G. (1995). Executive system dysfunction in the aged monkey: Spatial and object reversal learning. *Neurobiology of Aging*, *16*, 947–954.
- Lenth, R. (2020). *emmeans: Estimated marginal means, aka least-squares means* (Version 1.4.7). <https://CRAN.R-project.org/package=emmeans>
- Lewis, J.L., & Kamil, A.C. (2006). Interference effects in the memory for serially presented locations in Clark's nutcrackers, *Nucifraga columbiana*. *Journal of Experimental Psychology: Animal Behavior Processes*, *32*, 407–418. <https://doi.org/10.1037/0097-7403.32.4.407>
- Lewis, J.L., Kamil, A.C., & Webbink, K.E. (2013). Changing room cues reduces the effects on proactive interference in Clark's nutcrackers, *Nucifraga columbiana*. *Journal of Experimental Psychology*, *39*, 187–192. <https://doi.org/10.1037/a0031979>
- Liu, Y., Day, L.B., Summers, K., & Burmeister, S.S. (2016). Learning to learn: Advanced behavioural flexibility in a poison frog. *Animal Behaviour*, *111*, 167–172. <http://dx.doi.org/10.1016/j.anbehav.2015.10.018> 0003-3472/
- Ludecke, D. (2018). *ggeffects: Tidy data frames of marginal effects from regression models*. <http://dx.doi.org/10.21105/joss.00772> 1
- Ludecke, D., Makowski, D., & Waggoner, P. (2020). *performance: Assessment of regression models performance* (Version 0.4.4). <https://CRAN.R-project.org/package=performance>
- Mackintosh, J.J., & Cauty, A. (1971). Spatial reversal learning in rats, pigeons, and goldfish. *Science*, *22*, 281–282.
- Madden, J.R., Langley, E.J.G., Whiteside, M.A., Beardsworth, C.E., & van Horik, J.O. (2018). The quick are the dead: Pheasants that are slow to reverse a learned association survive for longer in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*, Article 20170297.
- Meigs, J.B., Smith, D.C., & Van Buskirk, J. (1983). Age determination of black-capped chickadees. *Journal of Field Ornithology*, *54*(3), 283–286.

- Mistlberger, R.E. (2009). Food-anticipatory circadian rhythms: Concepts and methods. *European Journal of Neuroscience*, *30*, 1718–1729.
- Murphy, R.A., Mondragon, E., & Murphy, V.A. (2008). Rule learning by rats. *Science*, *319*, 1849–1851. <https://doi.org/10.1126/science.1151564>
- Nakagawa, S., & Cuthill, I.C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews*, *82*, 591–605.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Pavlov, I.P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford University Press.
- Pitera, A.M., Branch, C.L., Bridge, E.S., & Pravosudov, V.V. (2018). Daily foraging routines in food-caching mountain chickadees are associated with variation in environmental harshness. *Animal Behaviour*, *143*, 93–104. <https://doi.org/10.1016/j.anbehav.2018.07.011>
- Pyle, P. (1997). Molt limits in North American passerines. *North American Bird Bander*, *22*, 49–89.
- R Core Team. (2018). *R: A language and environment for statistical computing* (Version 3.5.2). R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rayburn-Reeves, R.M., Molet, M., & Zentall, T.R. (2011). Simultaneous discrimination reversal learning in pigeons and humans: Anticipatory and perseverative errors. *Learning & Behavior*, *39*(2), 125–137. <https://doi.org/10.3758/s13420-010-0011-5>
- Roberts, W.A. (2002). Are animals stuck in time? *Psychological Bulletin*, *128*(3), 473–489. <https://doi.org/10.1037/0033-2909.128.3.473>
- Roberts, W.A., & Feeney, M.C. (2009). The comparative study of mental time travel. *Trends in Cognitive Sciences*, *13*(6), 271–277. <https://doi.org/10.1016/j.tics.2009.03.003>
- Roberts, W.A., Feeney, M.C., MacPherson, K., Petter, M., McMillan, N., & Musolino, E. (2008). Episodic-like memory in rats: Is it based on when or how long ago? *Science*, *320*, 113–115. <https://doi.org/10.1126/science.1152709>
- Rodriguez, W.A., Borbely, L.S., & Garcia, R.S. (1993). Attenuation by contextual cues of retroactive interference of a conditional discrimination in rats. *Animal Learning & Behavior*, *21*, 101–105.

- Seu, E., Lang, A., Rivera, R.J., & Jentsch, J.D. (2009). Inhibition of the norepinephrine transporter improves behavioral flexibility in rats and monkeys. *Psychopharmacology*, *202*, 505–519. <https://doi.org/10.1007/s00213-008-1250-4>
- Shettleworth, S.J. (2010). *Cognition, evolution, and behavior* (2nd ed.). Oxford University Press.
- Shimp, C.P. (1976). Short-term memory in the pigeon: Relative recency. *Journal of Experimental Analysis of Behavior*, *25*, 55–61.
- Shimp, C.P., & Moffitt, M. (1974). Short-term memory in the pigeon: Stimulus–response associations. *Journal of Experimental Analysis of Behavior*, *22*, 507–512.
- Schielzeth, H., Dingemans, N.J., Nakagawa, S., Westneat, D.F., Algue, H., Teplitsky, C., Reale, D., Dochtermann, N.A., Garamszegi, L.Z., & Araya-Ajoy, Y.G. (2020). Robustness of linear mixed-effects models to violations of distributional assumptions. *Methods in Ecology and Evolution*, *11*(9), 1141–1152. <https://doi.org/10.1111/2041-210X.13434>
- Schielzeth, H., & Forstmeier, W. (2009). Conclusions beyond support: Overconfident estimates in mixed models. *Behavioral Ecology*, *20*(2), 416–420. <https://doi.org/10.1093/beheco/arn145>
- Sonnenberg, B.R., Branch, C.L., Pitera, A.M., Bridge, E.S., & Pravosudov, V.V. (2019). Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, *29*(4), 670–676. <https://doi.org/10.1016/j.cub.2019.01.006>
- Stagner, J.P., Michler, D.M., Rayburn-Reeves, R.M., Laude, J.R., & Zentall, T.R. (2013). Midsession reversal learning: Why do pigeons anticipate and persevere? *Learning & Behavior*, *41*, 54–60. doi:10.3758/s13420-012-0077-3
- Stanton, L.A., Bridge, E.S., Huizinga, J., Johnson, S.R., Young, J.K., & Benson-Amram, S. (2021). Variation in reversal learning by three generalist mesocarnivores. *Animal Cognition*, *24*, 555–568. <https://doi.org/10.1007/s10071-020-01438-4>
- Stephens, D.W. (1991). Change, regularity, and value in the evolution of animal learning. *Behavioral Ecology*, *2*, 77–89. <https://doi.org/10.1093/beheco/2.1.77>
- Strang, C.G., & Sherry, D.F. (2014). Serial reversal learning in bumblebees (*Bombus impatiens*). *Animal Cognition*, *17*(3), 723–734. <https://doi.org/10.1007/s10071-013-07041>
- Suddendorf, T., & Corbalis, M.C. (2007). The evolution of foresight: What is mental time travel, and is it unique to humans? *Behavioral and Brain Sciences*, *30*, 299–351. <https://doi.org/10.1017/S0140525X07001975>

- Tello-Ramos, M.C., Branch, C.L., Kozlovsky, D.Y., Pitera, A.M., & Pravosudov, V.V. (2019). Spatial memory and cognitive flexibility trade-offs: To be or not to be flexible, that is the question. *Animal Behaviour*, *147*, 129–136. <https://doi.org/10.1016/j.anbehav.2018.02.019>
- Tello-Ramos, M.C., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., & Pravosudov, V.V. (2018). Memory in wild mountain chickadees from different elevations: Comparing first-year birds with older survivors. *Animal Behaviour*, *137*, 149–160. <https://doi.org/10.1016/j.anbehav.2017.12.019>
- Tille, Y., Newman, J.A., Healy, S.D. (1996). New tests for departures from random behavior in spatial memory experiments. *Animal Learning and Behavior*, *24*, 327–340.
- Tulving, E. (1985). Memory and consciousness. *Canadian Psychology/Psychologie Canadienne*, *26*, 1–12.
- van Horik, J.O., Beardsworth, C.E., Laker, P.R., Langley, E.J.G., Whiteside, M.A., & Madden, J.R. (2019). Unpredictable environments enhance inhibitory control in pheasants. *Animal Cognition*, *22*, 1105–1114.
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.
- Wixted, J.T. (2004). The psychology and neuroscience of forgetting. *Annual Review of Psychology*, *55*, 235–269.

Tables

Table 4.1. Term definitions

Table 4.1

Term	Definition
Trial	Starts when a bird visits a feeder at an array; ends when the bird visits the correct rewarding feeder
Reversal	When the rewarding feeder switches locations every day
Reversal feeder	The feeder that provided a food reward during the previous reversal but is no longer rewarding during the current reversal
Location errors	The number of feeders visited that did not provide a food reward (maximum of 7) per trial. The sum of neutral errors per trial and the reversal error (only one possible per trial)
Reversal errors	An incorrect visit to the reversal feeder; maximum of one per trial
Neutral errors	The number of feeders visited that never provided a food reward during the reversal task; maximum of six per trial
Performance	Generic term to describe the number of errors in a given context, e.g., location errors. Better performance describes fewer errors
Single reversal ability	Performance on the 5-day single reversal task before the first spatial serial reversal (Serial R1), used to assess cognitive flexibility before the serial reversal task
Reversal rule	The “win-stay lose-shift” strategy: individuals learn that if food is not present in location A, they must shift to location D. Learning this rule perfectly should be reflected in “one-trial, one-error” learning, in which individuals make one reversal error and no neutral errors in a given trial
Ability to predict	Individuals learn that the reversal rule is associated with changing days, and they make no errors in the first trial each day, going directly to the correct feeder

Table 4.2. Serial R1 GLMM model results with location errors per trial during trials 1–5

Table 4.2

Focal Variable	Effect	Spatial Serial Reversal 1 (N = 97)							
		Trial 1				Trials 2–5			
		Estimate	SE	χ^2	P	Estimate	SE	χ^2	P
Spatial learning and memory	Day	-0.18	0.04	16.52	<0.001	-0.01	0.06	0.01	0.92
	Elevation	0.32	0.08	15.17	<0.001	—	0.13	0.17	0.68
	Spatial learning and memory	0.07	0.04	3.38	0.07	0.05	0.06	0.62	0.43
	Previous reversal trials	< 0.001	0.07	0.00	0.99	-0.25	0.1	6.22	0.01
	Trial	—	—	—	—	—	—	11.83 ^a	0.008
	Day* elevation	0.17	0.06	8.05	0.005	-0.05	0.09	0.34	0.56
	Bird ID ^b	—	—	—	—	—	—	—	—
				$\sigma^2 = 0.08$				$\sigma^2 = 0.21$	
				$R^2c = 0.25,$				$R^2c = 0.13,$	
				$R^2m = 0.09$				$R^2m = 0.02$	
				AIC = 2034.1				AIC = 2989.7	
Single reversal ability	Day	-0.18	0.04	15.81	<0.001	-0.01	0.06	0.01	0.94
	Elevation	0.32	0.07	18.76	<0.001	0.01	0.12	0.01	0.92
	Single reversal ability	0.25	0.04	31.96	<0.001	-0.11	0.07	2.07	0.15
	Previous reversal trials	0.07	0.06	1.10	0.29	-0.22	0.1	5.07	0.02
	Trial	—	—	—	—	—	—	11.77 ^a	0.008
	Day* elevation	0.15	0.05	6.68	0.01	-0.06	0.09	0.41	0.52
	Single reversal ability* elevation	-0.19	0.08	6.26	0.01	0.44	0.13	11.76	<0.001
Bird ID ^b	—	—	—	—	—	—	—	—	
				$\sigma^2 = 0.05$				$\sigma^2 = 0.17$	
				$R^2c = 0.26,$				$R^2c = 0.1,$	
				$R^2m = 0.17$				$R^2m = 0.04$	
				AIC = 2010.8				AIC = 2981.1	

SE, Standard Error. Focal variable refers to the fixed effect of primary interest for the analysis. Bolded values indicate statistical significance ($\alpha < 0.05$). Separate models were fitted for spatial learning and memory and single reversal ability and trial 1 and trials 2–5. Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth, (2013). Unstandardized regression coefficients used as estimates. Estimates (Est.) for elevation are relative to high elevation. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

^bBird ID fit as random intercept.

Table 4.3. Model results for the total errors in the last five trials in Serial R1 and R2

Table 4.3

Effect	Serial R1 (N = 97)				Serial R2 (N = 235)			
	Est.	SE	χ^2_1	P	Est.	SE	χ^2_1	P
Day	-0.04	0.09	0.24	0.63	-0.17	0.03	24.33	< 0.001
Elevation	0.35	0.14	6.32	0.01	-0.15	0.09	2.66	0.10
Total daily trials	-0.42	0.11	14.43	< 0.001	-0.37	0.06	40.11	< 0.001
Day*elevation	-0.08	0.11	0.59	0.44	0.01	0.06	0.01	0.90
Bird ID ^a	—	—	—	$\sigma^2 = 0.13$ $R^2c = 0.15$, $R^2m = 0.06$	—	—	—	$\sigma^2 = 0.22$ $R^2c = 0.20$, $R^2m = 0.05$

SE, Standard Error. Bolded values indicate statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth (2013). Unstandardized regression coefficients used as estimates (Est.). Elevation Est. are relative to high elevation in Serial R1 and low elevation in Serial R2.

^aBird ID fitted as a random intercept.

Table 4.4. Logistic GLMM results for the odds of making a reversal error during trial 1 each day of Serial R1 and Serial R2

Table 4.4

Effect	Spatial Serial Reversal 1 (N = 97)				Spatial Serial Reversal 2 (N = 180, N = 164 ^a)			
	Estimate	SE	χ^2_1	P	Estimate	SE	χ^2_1	P
Focal variable: Spatial learning and memory	0.1	0.13	0.58	0.45	-0.15	0.06	7.07	0.008
Day	-0.51	0.19	7.28	0.007	-0.03	0.1	0.09	0.77
Elevation	0.01	0.27	0.002	0.97	-0.29	0.13	4.70	0.03
Previous reversal trials	0.65	0.25	6.82	0.009	0.39	0.12	10.01	0.002
Day*elevation	0.73	0.27	7.33	0.007	0.16	0.12	1.68	0.19
Bird ID ^b	—	—		$\sigma^2 < 0.01$	—	—		$\sigma^2 = 0.05$
				$R^2c = 0.09$				$R^2c = 0.05$
				$R^2m = 0.09$				$R^2m = 0.03$
				AIC = 444.6				AIC = 2079.9
Focal variable: Single reversal ability	0.93	0.23	16.38	<0.001	0.03	0.06	0.17	0.68
Day	-0.54	0.2	7.38	0.007	-0.02	0.11	0.03	0.86
Elevation	-0.32	0.32	0.97	0.32	-0.22	0.14	2.41	0.12
Previous reversal trials	0.79	0.27	8.66	0.003	0.34	0.13	7.30	0.007
Day*elevation	0.73	0.28	6.97	0.008	0.15	0.13	1.37	0.24
Single reversal ability*elevation	-1.35	0.31	18.58	<0.001			NS	
Bird ID ^b	—	—		$\sigma^2 < 0.01$	—	—		$\sigma^2 = 0.08$
				Singular fit				$R^2c = 0.04$
								$R^2m = 0.02$
				AIC = 420.9				AIC = 1928.3

Est., Unstandardized regression coefficient estimate; NS, Nonsignificant; SE, Standard Error. Focal variable refers to the fixed effect of primary interest for the analysis. Bolded values indicate statistical significance ($\alpha < 0.05$). Separate models were fitted for spatial learning and memory and single reversal ability. Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth, (2013). “NS” indicates nonsignificant term removed from final model. Estimates (Est.) on the log-odds scale. Elevation estimates relative to high elevation.

^aModels fitted with spatial learning and memory for Serial R2 had a sample size of 180; models fitted with single reversal ability for Serial R2 had a sample size of 164.

^bBird ID fitted as a random intercept.

Table 4.5. Serial R2 model results for the number of location errors in trials 1–5

Table 4.5

Effect	Spatial Serial Reversal 2							
	Trial 1				Trials 2–5			
	Estimate	SE	χ^2	<i>P</i>	Estimate	SE	χ^2	<i>P</i>
Focal variable: Spatial learning and memory	-0.01	0.03	0.34	0.56	0.07	0.04	2.68	0.10
Day	-0.23	0.02	92.66	< 0.001	-0.59	0.04	216.45	< 0.001
Elevation	0.03	0.04	0.67	0.41	0.29	0.09	10.37	0.001
Trials previous day	0.1	0.02	11.53	< 0.001	0.22	0.05	18.36	< 0.001
Trial	—	—	—	—	—	—	16.90 ^a	< 0.001
Day*elevation	0.07	0.03	5.70	0.02	0.36	0.05	54.68	< 0.001
Bird ID ^b	—	—	$\sigma^2 = 0.02$ $R^2c = 0.16$ $R^2m = 0.09$ AIC = 8473.2		—	—	$\sigma^2 = 0.22$ $R^2c = 0.20$ $R^2m = 0.09$ AIC = 12914.9	
Focal Variable: Single reversal ability	0.02	0.03	0.80	0.37	-0.01	0.04	0.08	0.78
Day	-0.23	0.03	81.33	< 0.001	-0.61	0.04	190.07	< 0.001
Trials previous day	0.1	0.02	9.22	0.002	0.22	0.05	17.37	< 0.001
Trial	—	—	—	—	—	—	15.19 ^a	0.002
Day*elevation	0.07	0.03	5.32	0.02	0.38	0.05	54.80	< 0.001
Bird ID ^b	—	—	$\sigma^2 = 0.03$ $R^2c = 0.16$ $R^2m = 0.09$ AIC = 7753.5		—	—	$\sigma^2 = 0.22$ $R^2c = 0.20$ $R^2m = 0.09$ AIC = 11777.9	

Estim., Unstandardized regression coefficient estimate. SE, Standard Error. Focal variable refers to primary fixed effect of interest. Bolded values indicate statistical significance ($\alpha < 0.05$). Separate models were fitted for spatial learning and memory ($N = 180$) and single reversal ability ($N = 164$), due to possible covariance. Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth (2013). Elevation estimates are relative to high elevation. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

^bBird ID fitted as a random intercept.

Table 4.6. GLMM model results comparing location errors in trial 1 of Serial R2 based on participation in Serial R1 and R2

Table 4.6

Data set	Comparing Serial R1 and R2				
	Effects	Estimate	SE	χ^2_1	<i>P</i>
Full participation in Serial R1 and R2 or only full participation in Serial R2 (<i>N</i> = 119)	Day	-0.23	0.03	80.31	<0.001
	Elevation	<0.001	0.04	0.001	0.99
	SR1 Participation (full)	0.13	0.05	7.69	0.006
	Previous reversal trials	0.11	0.04	9.69	0.002
	Day*elevation	0.07	0.03	4.07	0.04
	Bird ID ^a	—	—	—	Var. = 0.02
					<i>R</i> ² c = 0.15 <i>R</i> ² m = 0.10
Full participation in both Serial R1 and R2 (<i>N</i> = 86)	Day	-0.13	0.02	34.41	<0.001
	Elevation	-0.37	0.07	28.14	<0.001
	Task (Serial R1 or R2)	0.14	0.05	9.39	0.002
	Previous reversal trials	0.09	0.04	6.93	0.008
	Day*elevation	-0.08	0.03	4.92	0.03
	Task*elevation	0.38	0.1	26.80	<0.001
	Bird ID ^a	—	—	—	Var. = 0.02 <i>R</i> ² c = 0.16 <i>R</i> ² m = 0.11

SE, Standard Error. Bolded values indicate statistical significance ($\alpha < 0.05$). Conditional and marginal *R*² (*R*²c and *R*²m) were calculated according to Nakagawa and Schielzeth (2013). Elevation estimates are relative to high elevation.

^aBird ID fitted as a random intercept.

Table 4.7. Sum of reversal errors in the first 20 trials

Table 4.7

Response variable	Effects	Estimate	SE	χ^2_1	P
Serial R1: Sum of reversal errors in the first 20 trials per day (N = 97)	Elevation	0.13	0.07	3.44	0.06
	Day	-0.03	0.04	0.62	0.43
	Previous reversal trials	-0.02	0.06	0.09	0.77
	Elevation*day	0.01	0.06	0.01	0.91
	Bird ID ^a	—	—	—	$\sigma^2 = 0.046$
					$R^2c = 0.125$
					$R^2m = 0.013$
					AIC = 1987.9
Serial R2: Sum of reversal errors in first 20 trials per day (N = 86)	Elevation	0.24	0.047	25.91	< 0.001
	Days	-0.12	0.036	10.69	0.001
	Previous reversal trials	0.11	0.046	5.72	0.02
	Elevation*day	0.1	0.046	4.94	0.03
	Bird ID ^a	—	—	—	$\sigma^2 = 0.003$
					$R^2c = 0.05$
					$R^2m = 0.05$
					AIC = 3294.7
Serial R2: Sum of reversal errors to Serial R1 locations in first 20 trials per day (N = 86)	Elevation	-0.01	0.12	0.003	0.95
	Day	-0.49	0.04	151.48	< 0.001
	Previous reversal trials	0.12	0.06	3.79	0.05
	Elevation*day	0.18	0.06	10.92	< 0.001
	Bird ID ^a	—	—	—	$\sigma^2 = 0.23$
					$R^2c = 0.42$
					$R^2m = 0.17$
					AIC = 3563.6

SE, Standard Error. Bolded values indicate statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth (2013). Unstandardized regression coefficients used as estimates. Elevation estimates are relative to low elevation for Serial R1 and high elevation for Serial R2. For Serial R2, only including birds that fully participated in both Serial R1 and R2 (N = 86).

^aBird ID fitted as a random intercept.

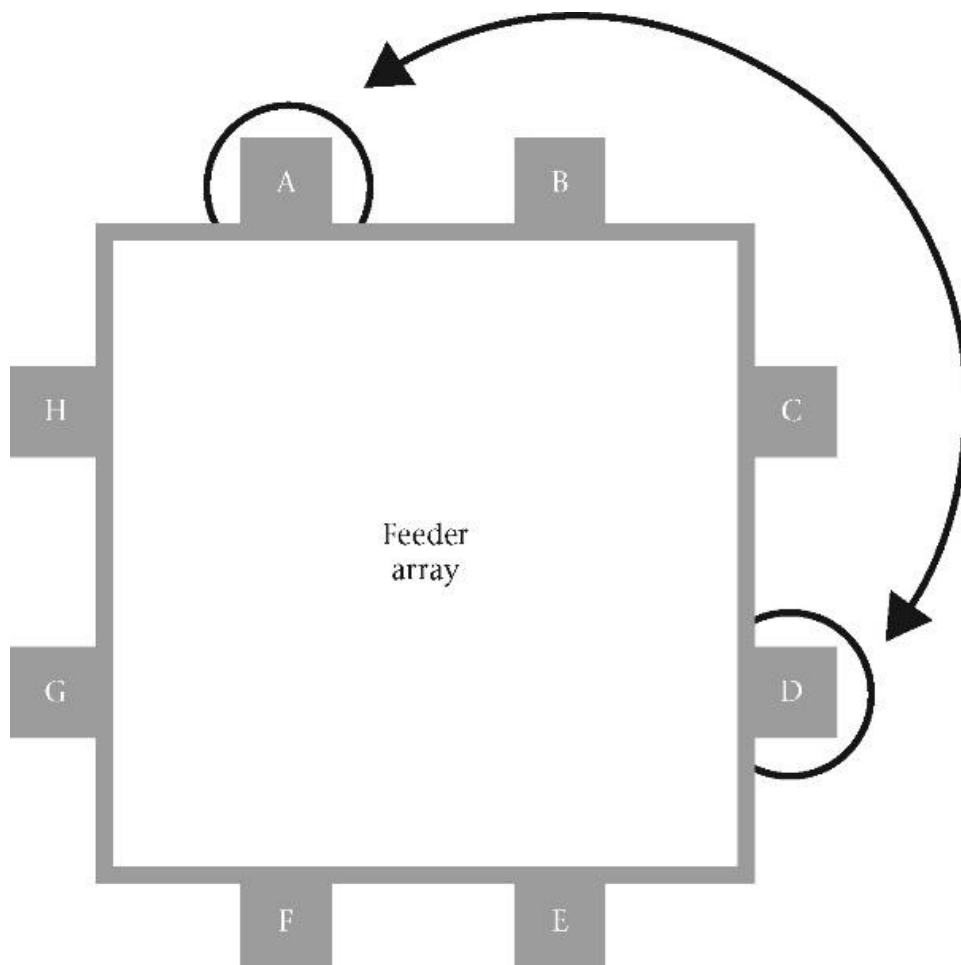
Figures*Figure 4.1*

Figure 4.1. Example experimental set-up using a feeder array. Eight feeders (grey squares) were arranged on a square frame. For each bird, the rewarding feeder switched each day between two of the eight possible feeders (for example, feeder A and feeder D). Following Croston et al., (2016). Individuals were not all assigned to the same feeders.

Figure 4.2

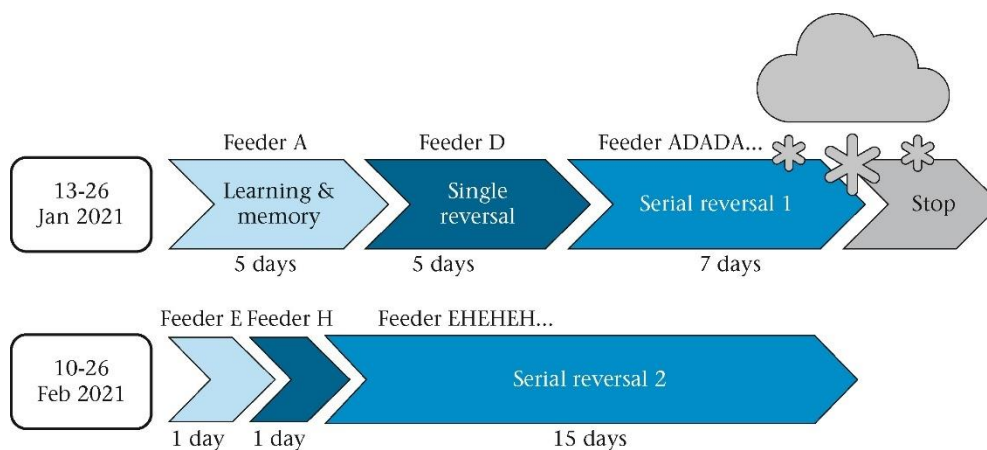


Figure 4.2. Timeline of experiments. During 13–26 January 2021, three cognitive tasks were conducted: (1) 5-day learning and memory task with birds assigned to feeder A; (2) 5-day single reversal task with birds assigned to feeder D; (3) 7-day serial reversal 1 task with feeder assignments alternating daily, “ADADA...”. Experiments stopped during a snowstorm. During 10–26 February 2021, birds were given (1) 1 day to learn feeder E, (2) 1 day to learn feeder H and (3) a 15-day serial reversal task 2 with feeder assignments alternating daily, “EHEHE...”.

Figure 4.3

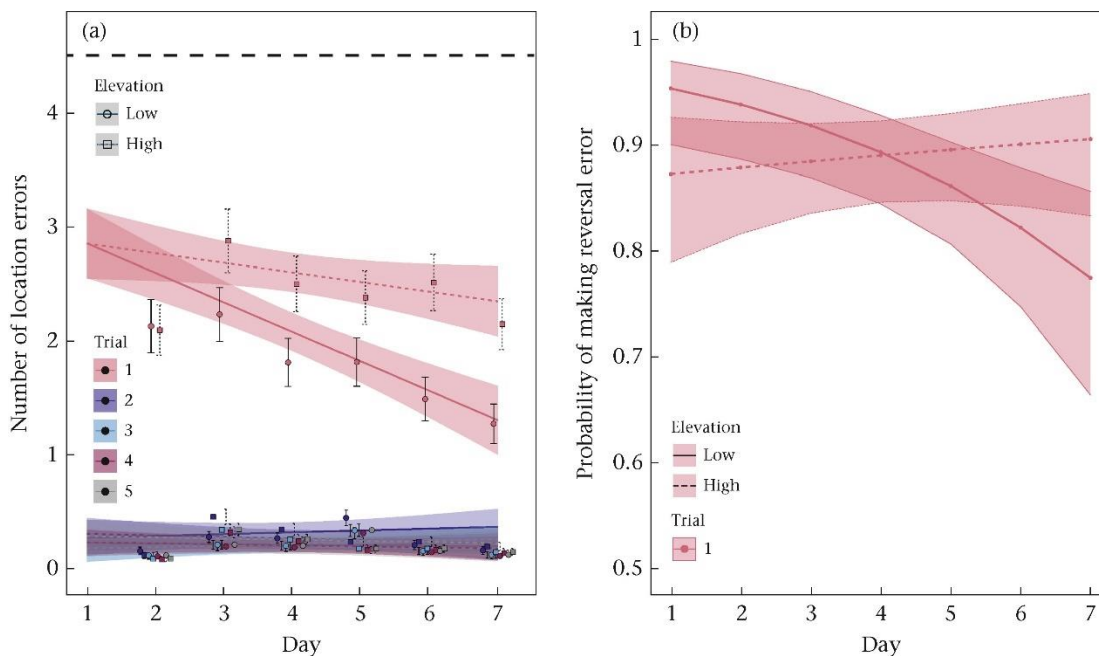


Figure 4.3. For Serial R1, (a) the number of location errors per trial for trials 1–5 per day and (b) the predicted probability of visiting the previous feeder (i.e., make a reversal error) in trial 1 per day. Shaded areas indicate 95% confidence intervals generated using the “ggplot lm” method. In (a), points and error lines indicate estimated marginal means and standard errors were calculated from reported GLMMs. Heavy dashed lines indicate expected random error values. In (b), the predicted probability was calculated using reported GLMMs and “ggeffects”.

Figure 4.4

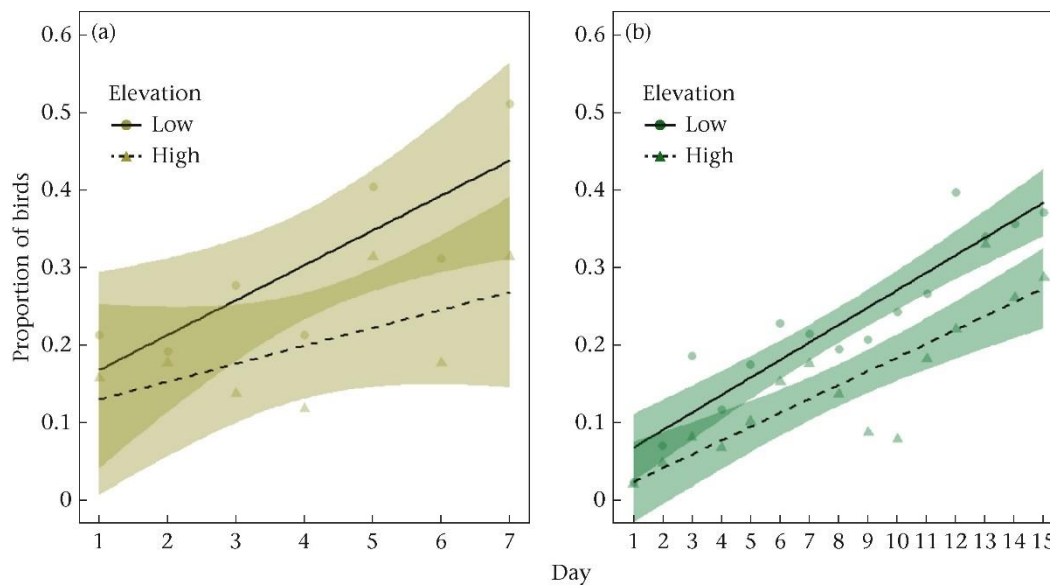


Figure 4.4. Proportion of birds that showed “one-trial, one-error” learning (one reversal error, no neutral errors) during trial 1 of (a) Serial R1 and (b) Serial R2. Lines represent linear regression and shaded areas indicate standard error of the regression line.

Figure 4.5

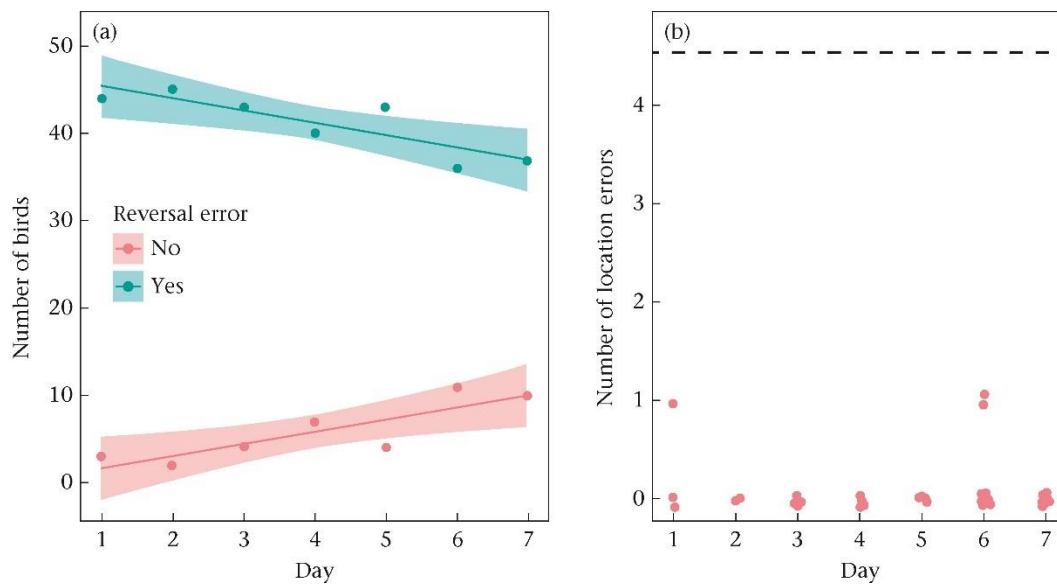


Figure 4.5. At low elevation in Serial R1: (a) the number of birds per day that either made or did not make a reversal error during trial 1 after each reversal and (b) the number of neutral errors made by birds that did not make a reversal error during trial 1.

Figure 4.6

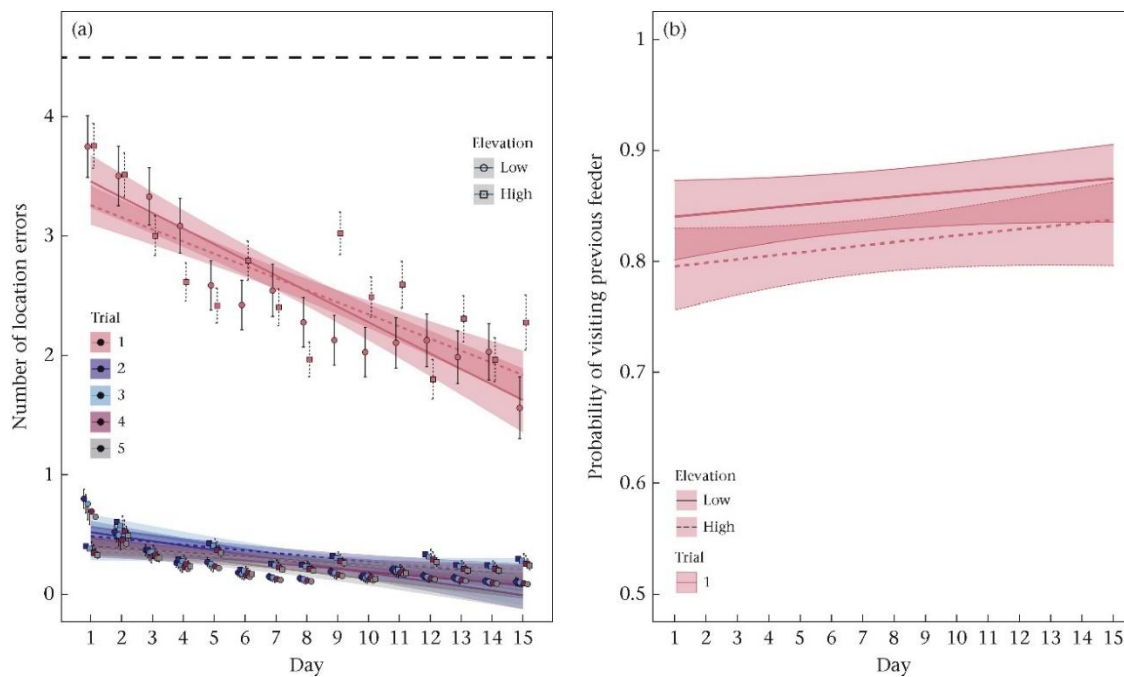


Figure 4.6. For Serial R2, (a) the number of location errors per trial for trials 1–5 per day and (b) the probability of visiting the previous feeder (i.e., making a reversal error) in trial 1 per day. Confidence intervals (shaded) generated from “ggplot lm” method. Estimated marginal means (points) and standard errors (lines) were generated from reported GLMMs. Heavy dashed line indicates expected random value. In (b), predicted probabilities were simulated using GLMMs and “ggeffects”.

Figure 4.7

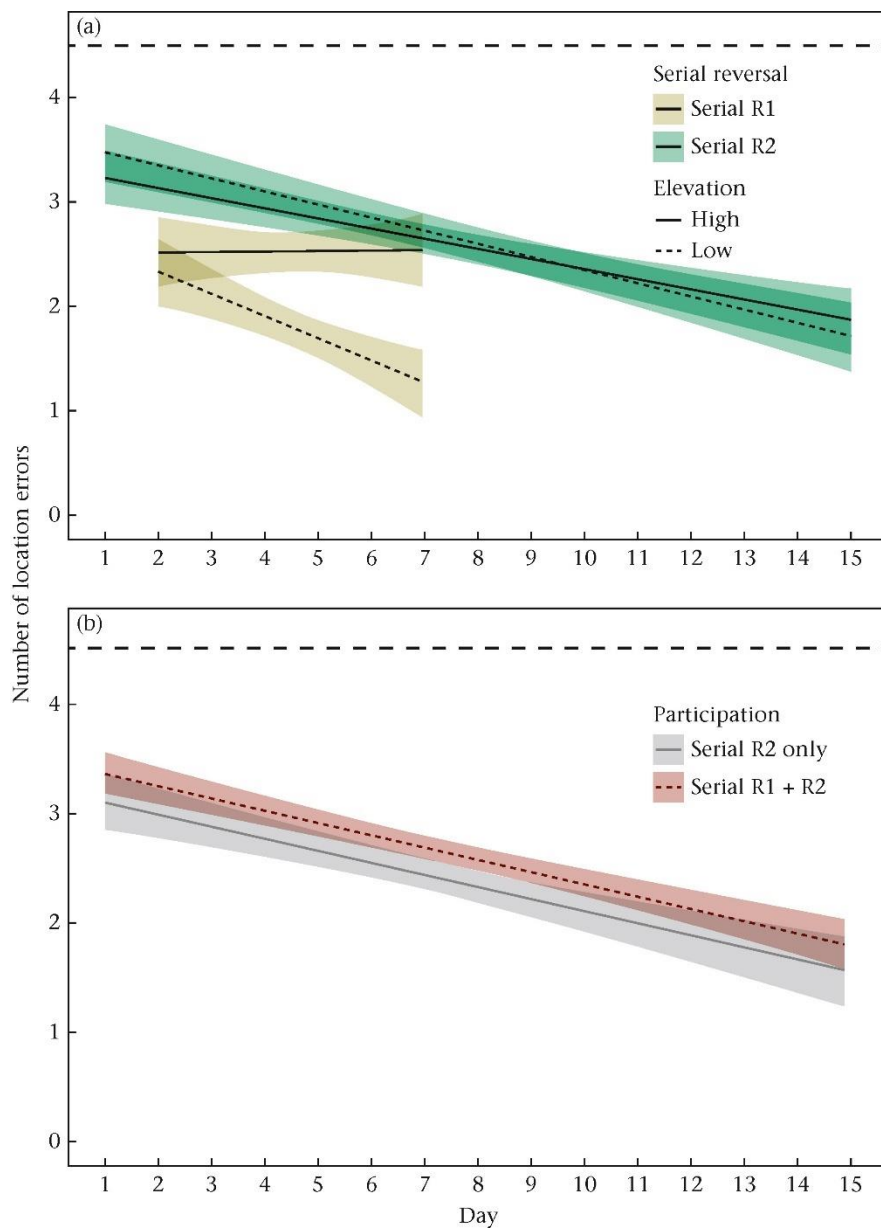


Figure 4.7. Performance (location errors) in trial 1 each day, comparing Serial R1 and R2, for (a) birds that participated in both Serial R1 and R2 ($N = 86$) by elevation and serial reversal task and (b) birds that participated in both Serial R1 and R2 ($N = 86$) compared to birds that only participated in Serial R2 ($N = 33$) by elevation. In (b), only Serial R2 performance is depicted. Heavy dashed line indicates expected random values.

Figure 4.8

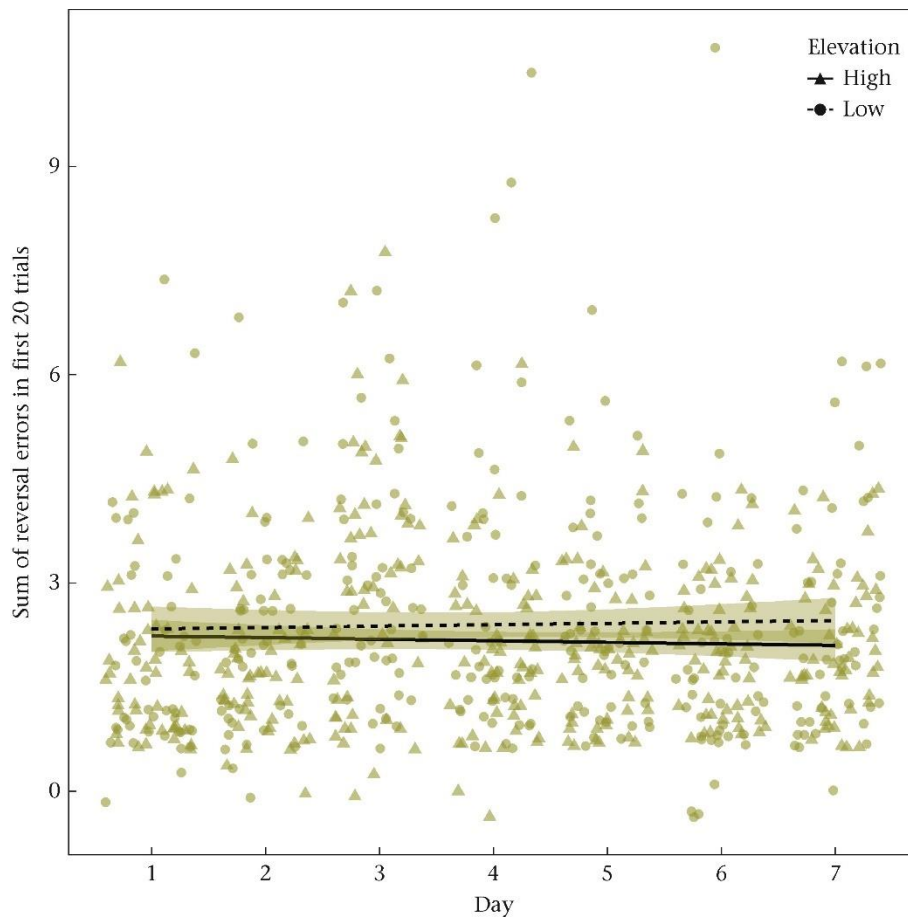


Figure 4.8. Sum of reversal errors in the first 20 trials per day of Serial R1 by day and elevation. All reversal errors were made during Serial R1. Includes only birds that participated in Serial R1 and the spatial learning and memory task and single reversal learning task ($N = 97$).

Figure 4.9

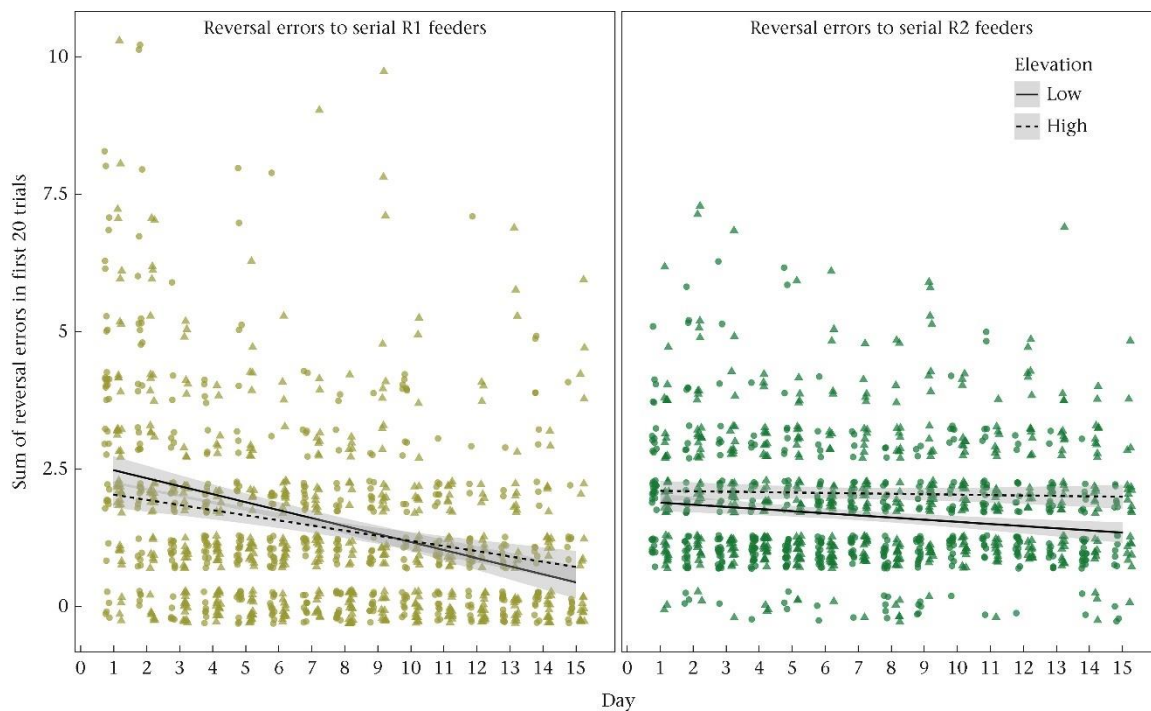


Figure 4.9. Sum of reversal errors in the first 20 trials per day of Serial R2 by day elevation (high: triangles; low: circles) and reversal error type. All reversal errors were made during trials in Serial R2, separated by type: reversal errors to Serial R1 target feeders (yellow) and reversal errors made to the Serial R2 target feeders (green). Includes only birds that participated in Serial R1 and Serial R2 and the spatial learning and memory task and the single reversal learning task ($N = 86$).

Appendix

Singular Fit Justification and Discussion

In this study, we present a generalized linear mixed-effects model (GLMM) with a singular fit and use the results for interpretation. The singular fit warning from the R package “lme4” indicates that “some “dimensions” of the variance–covariance matrix have been estimated as exactly zero” (Bates et al., 2015). Here, we include a brief discussion of why we used this model despite the warning and some potential pitfalls.

The singular fit model (Main Text, Table 4.4) and two associated post hoc GLMM models were used as part of a confirmatory hypothesis testing approach. We expected the probability that a chickadee made a reversal error during the first trial of each day to vary with reversal learning ability across days in the first serial reversal experiment (Serial R1). Thus, we fitted a GLMM (binomial distribution, logit link, bobyqa optimizer) with the following focal fixed effects: day, single reversal learning ability (determined from performance on a previous cognitive task) and an interaction of day with single reversal learning ability. We added the following fixed effects to control for other variation in the data: the number of trials completed the previous day and an interaction between day and elevation. Most importantly for this discussion, we fitted a random intercept of individual bird to control for pseudoreplication, as the data set comprised 97 birds observed six times (once per day).

There are several potential causes of a singular fit warning from “lme4”. The model may be overfitted from including too many variables, or the random effects may be improperly specified. Importantly, the authors of “lme4” specifically allowed the

software to report singular fit models because these models can still provide insight into the trends of the data (Bates et al., 2015). To explore this, we used a stepwise approach to remove variables from the model and evaluate the variance of the random effects.

We determined that the model was not overfit because of too many parameters (i.e., including interaction effects of day*elevation and the fixed effect of the total trials completed the previous day). Instead, the warning appeared to be the result of covariance between the random intercept (individual bird) and the cognitive variable we included (single reversal learning ability). In other words, the variation in the response variable (whether or not the bird made a reversal error in the first trial of each day) that was explained by the random intercept (individual bird) was almost entirely explained by the fixed effect of single reversal learning ability, resulting in a zero variance estimate for the random effect. However, we could not remove the random effect, due to pseudoreplication issues, and we could not remove the focal fixed effect of single reversal learning ability. Furthermore, this singular fit is harmonious with our overall hypotheses: we expected that better performance on a single reversal learning task would correlate with lower odds of visiting the previous day's rewarding feeder on a serial reversal learning task, suggesting that birds were better able to switch to the new feeder without relying on memories of the previous feeder location.

Finally, there are several studies that suggest linear mixed-effects models are highly robust and that many issues with assumptions and fitting can be resolved through analyzing the results within an ecological context (Schielzeth et al., 2020). Thus, we have chosen to include this singular fit model and additionally include the variance of the

random intercepts in the main text so the reader can assess the data and see the trends, in addition to the shortcomings of the model. As the purpose of this study was to explore specific hypotheses and compare fixed effects, we chose to fit linear models to approximate differences in learning between individuals, rather than fit nonlinear learning curves to best describe learning on the serial reversal tasks. Thus, we feel confident reporting this singular fit model and the two singular fit post hoc models associated with it.

There are several possible pitfalls to using this model for interpretation. First, models with random intercepts (such as the singular fit model we report) have been shown to increase type I error in comparison to models fitted with random slopes (e.g., Schielzeth & Forstmeier, 2008). But we did not reformat our random effects structure because the random intercept more appropriately fit our a priori hypotheses, and because one approach to reduce singular fit is to simplify the random effects. This usually involves removing random slope effects first, which we had already done in our models. Second, another approach is to “keep it maximal”, or keep as many effects as possible without leading to a singular fit (Barr et al., 2013). One advantage of this approach is maximally fit models often perform strongly and have strong power. Thus, although we could not remove the variable that produced the singular fit, we kept as many variables as we felt appropriate to support the hypothesis in question. Finally, in general, the key issue with singular fit models (also called overfitting) is that while they tightly describe the sample of data, they generally do not predict future data and trends as well. This suggests that interpretations of this model might not apply to the overall population, only to our sample. In our case, the model suggests that birds with worse single reversal learning

ability were more likely to visit the previous feeder in the first trial each day of Serial R1, and that this effect varied between elevations. We also present a similar model with a different cognitive ability, and so we report that the noncognitive fixed effects in both models are highly consistent. This gives us additional confidence that the singular fit model likely also appropriately estimates the effect of reversal learning ability. We feel that this approach balances the risk of a singular fit model appropriately and thus we include it in the main manuscript.

Appendix Tables

Table A4.1. Excluded birds by elevation and serial reversal task

Data set	Serial R1			Serial R2			Combined Serial R1 + Serial R2		
	High	Low	Total	High	Low	Total	High	Low	Total
Before exclusions	168	104	272	194	125	319	196	125	321
After exclusions	51	47	98	149	86	235	154	92	246
Number excluded	117	57	174	45	39	84	42	33	75

Serial R1, Serial Reversal Learning Task 1; Serial R2, Serial Reversal Learning Task 2.

Table A4.2. Models using trial 1 performance from all 7 days of Serial R1

Effect	Location errors			Probability of visiting previous feeder		
	Estimate	χ^2_1	<i>P</i>	Estimate	χ^2_1	<i>P</i>
Focal variable: Spatial learning and memory	0.07	3.30	0.07	0.11	0.68	0.41
Day	-0.26	42.30	<0.001	-0.59	10.16	0.001
Elevation	—	12.92	<0.001	—	0.001	0.98
Day*elevation	0.19	14.04	<0.001	0.70	7.88	0.005
		$R^2c = 0.28$			$R^2c = 0.09$	
		$R^2m = 0.11$			$R^2m = 0.05$	
Focal variable: Single reversal ability	0.23	31.00	<0.001	0.84	15.93	<0.001
Day	-0.26	42.14	<0.001	-0.62	10.64	0.001
Elevation	—	15.97	<0.001	—	1.04	0.31
Day*elevation	0.19	13.99	<0.001	0.73	8.33	0.004
Single reversal*elevation	-0.18	6.06	0.01	-1.27	18.57	<0.001
		$R^2c = 0.29$			Singular fit	
		$R^2m = 0.18$				

Focal variable refers to the fixed effect of primary interest for the analysis. Bold text indicates statistical significance ($\alpha < 0.05$). Models do not control for the number of trials completed the previous day (one model for each cognitive task). Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth, (2013). Unstandardized regression coefficients used as estimates, reported on log-odds scale for the probability of visiting previous feeder.

Table A4.3. Location errors during Trials 1-5 in Serial R1 with age as a predictor, included as categorical age (juvenile versus adult) or numeric age (years).

Effect	Location errors in trial 1			Location errors in trials 2-5		
	Estimate	χ^2_1	<i>P</i>	Estimate	χ^2	<i>P</i>
Focal variable: Age, categorical	—	0.05	0.82	—	0.56	0.45
Day	-0.18	16.30	0.001	-0.01	0.03	0.87
Elevation	—	12.49	0.001	—	0.07	0.79
Trials previous day	0.01	0.03	0.87	-0.23	5.01	0.03
Trial	—	—	—	—	7.25 ^a	0.007
Day*elevation	0.16	7.58	0.006	-0.05	0.33	0.56
			$R^2_c = 0.25$ $R^2_m = 0.07$			$R^2_c = 0.13$ $R^2_m = 0.02$
Focal variable: Age, years	-0.03	0.36	0.55	-0.01	0.03	0.85
Day	-0.18	16.36	0.001	-0.01	0.03	0.85
Elevation	—	13.34	0.001	—	0.29	0.59
Trials previous day	0.01	0.01	0.94	-0.25	5.87	0.02
Trial	—	—	—	—	7.25 ^a	0.007
Day*elevation	0.16	7.71	0.005	-0.05	0.28	0.60
			$R^2_c = 0.25$ $R^2_m = 0.08$			$R^2_c = 0.13$ $R^2_m = 0.02$

Focal variable refers to the fixed effect of primary interest for the analysis. Bold text indicates statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2_c and R^2_m) were calculated according to Nakagawa and Schielzeth (2013). Unstandardized regression coefficients used as estimates. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

Table A4.4. Models for the probability of visiting the previous feeder and the number of neutral errors before visiting the previous feeder during Serial R1 with age as a predictor, included as categorical age (juvenile versus adult) or numeric age (years).

Effect	Probability of visiting the previous feeder in trial 1			Number of errors before visiting previous trial 1		
	Estimate	χ^2_1	<i>P</i>	Estimate	χ^2_1	<i>P</i>
Focal variable:	—	1.01	0.31	—	0.74	0.39
Age, categorical						
Day	-0.50	7.04	0.008	-0.21	0.32	0.57
Elevation	—	0.11	0.74	—	0.60	0.44
Trials previous day	0.74	8.19	0.004	-1.31	6.64	0.01
Trial	—	—	—	—	—	—
Day*elevation	0.70	6.86	0.009	0.42	0.78	0.38
			Singular fit			$R^2_c = 0.41$ $R^2_m = 0.11$
Focal variable:	-0.01	0.01	0.93	0.19	0.34	0.56
Age, years						
Day	-0.51	7.09	0.008	-0.20	0.33	0.57
Elevation	—	0.002	0.97	—	0.74	0.39
Trials previous day	0.66	6.55	0.01	-1.36	7.17	0.007
Trial	—	—	—	—	—	—
Day*elevation	0.72	7.13	0.008	0.44	0.83	0.36
			$R^2_c = 0.09$ $R^2_m = 0.09$			$R^2_c = 0.41$ $R^2_m = 0.10$

Focal variable refers to the fixed effect of primary interest for the analysis. Bold text indicates statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2_c and R^2_m) were calculated according to Nakagawa and Schielzeth (2013). Unstandardized regression coefficients used as estimates, reported on the log-odds scale for the probability of visiting the previous feeder in trial 1. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

Table A4.5 Location errors in trials 1-5 during Serial R2 with age.

Effect	Location errors in trial 1			Location errors in trials 2–5		
	Estimate	χ^2_1	<i>P</i>	Estimate	χ^2	<i>P</i>
Focal variable: Age, categorical	—	0.61	0.43	—	0.77	0.38
Day	-0.24	131.24	<0.001	-0.59	292.63	<0.001
Elevation	—	1.05	0.31	—	15.12	<0.001
Trials previous day	0.08	9.53	0.002	0.17	16.49	<0.001
Trial	—	—	—	—	55.52 ^a	<0.001
Day*elevation	0.08	8.66	0.003	0.36	73.89	<0.001
			<i>R</i> ² _c = 0.16 <i>R</i> ² _m = 0.09			<i>R</i> ² _c = 0.21 <i>R</i> ² _m = 0.10
Focal variable: Age, years	-0.02	1.26	0.26	-0.05	1.72	0.19
Day	-0.24	131.52	<0.001	-0.60	292.04	<0.001
Elevation	—	1.06	0.30	—	15.41	<0.001
Trials previous day	0.08	9.27	0.002	0.17	16.19	<0.001
Trial	—	—	—	—	55.51 ^a	<0.001
Day*elevation	0.08	8.75	0.003	0.36	74.10	<0.001
			<i>R</i> ² _c = 0.16 <i>R</i> ² _m = 0.09			<i>R</i> ² _c = 0.21 <i>R</i> ² _m = 0.10

Focal variable refers to the fixed effect of primary interest for the analysis. Bold text indicates statistical significance ($\alpha < 0.05$). Models use either categorical age (juvenile versus adult) or numeric age (in years). Conditional and marginal R^2 (R^2_c and R^2_m) were calculated according to Nakagawa and Schielzeth (2013). Top models include age (categorical) and bottom models include age (numeric). Unstandardized regression coefficients used as estimates. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

Table A4.6 Models for the probability of visiting the previous feeder and the number of location errors before visiting the previous feeder during Serial R2 with age.

Effect	Log-odds of visiting the previous feeder in trial 1			Number of errors when visiting previous feeder in trial 1		
	Estimate	χ^2_1	<i>P</i>	Estimate	χ^2_1	<i>P</i>
Focal variable:	—	0.20	0.65	—	1.04	0.31
Age, categorical						
Day	-0.06	0.37	0.54	-0.97	21.56	<0.001
Elevation	—	5.22	0.02	—	5.86	0.02
Trials previous day	0.51	23.53	<0.001	-0.12	0.57	0.45
Trial	—	—	—	—	—	—
Day*elevation	0.18	2.76	0.10	0.19	0.66	0.42
			<i>R</i> ² c = 0.06 <i>R</i> ² m = 0.03			<i>R</i> ² c = 0.30 <i>R</i> ² m = 0.20
Focal variable:	0.04	0.51	0.47	-0.01	0.02	0.87
Age, years						
Day	-0.05	0.36	0.55	-0.97	21.55	<0.001
Elevation	—	5.36	0.02	—	5.28	0.02
Trials previous day	0.51	24.20	<0.001	-0.10	0.35	0.55
Trial	—	—	—	—	—	—
Day*elevation	0.18	2.71	0.10	0.20	0.68	0.41
			<i>R</i> ² c = 0.06 <i>R</i> ² m = 0.03			<i>R</i> ² c = 0.30 <i>R</i> ² m = 0.20

Focal variable refers to the fixed effect of primary interest for the analysis. Bold text indicates statistical significance ($\alpha < 0.05$). Models use either categorical age (juvenile versus adult) or numeric age (in years). Conditional and marginal *R*² (*R*²c and *R*²m) were calculated according to Nakagawa and Schielzeth (2013). Top models include age (categorical) and bottom models include age (numeric). Unstandardized regression coefficients used as estimates. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

Table A4.7 Models results for Serial R1 with total neutral errors for trial 1 and trials 2–5

Response variable	Effect	Spatial Serial Reversal 1 (<i>N</i> = 97)			
		Estimate	SE	χ^2	<i>P</i>
Neutral errors in trial 1	Day	-0.24	0.06	16.54	< 0.001
	Elevation	0.48	0.12	0.83	< 0.001
	Spatial learning and memory	0.08	0.06	0.29	0.17
	Trials previous day	-0.07	0.09	0.66	0.42
	Day*elevation	0.21	0.08	0.14	0.006
	Bird ID ^b	—	—		Var. = 0.22
					<i>R</i> ² c = 0.34 <i>R</i> ² m = 0.10 AIC = 1790.08
Neutral errors in trials 2–5	Day	0.00	0.08	0.002	0.97
	Elevation	0.26	0.17	2.56	0.11
	Spatial learning and memory	0.03	0.08	0.16	0.69
	Trials previous day	-0.35	0.12	7.75	0.005
	Trial	—	—	0.62 ^a	0.09
	Day*elevation	-0.03	0.11	0.09	0.77
	Bird ID ^b	—	—		Var. = 0.33 <i>R</i> ² c = 0.15 <i>R</i> ² m = 0.03 AIC = 2094.53

SE, Standard Error. Bolded values indicate statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth (2013). Elevation estimates relative to high elevation. Unstandardized regression coefficients used as estimates. All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

^bBird ID fitted as random intercept.

Table A4.8. Model results for Serial R2 with total neutral errors for trial 1 and trials 2–5

Response variable	Effect	Spatial Serial Reversal 2 (N = 180)			
		Estimate	SE	χ^2	P
Neutral errors in trial 1	Day	-0.34	0.03	133.20	< 0.001
	Elevation	0.08	0.05	2.03	0.008
	Spatial learning and memory	0.00	0.03	0.01	0.94
	Trials previous day	0.11	0.04	8.00	0.01
	Day*elevation	0.10	0.04	7.34	0.007
	Bird ID ^b	—	—		Var. = 0.06 $R^2c = 0.24$ $R^2m = 0.13$ AIC = 7694.76
Neutral errors in trials 2–5	Day	-0.72	0.05	224.15	< 0.001
	Elevation	0.32	0.10	11.17	< 0.001
	Spatial learning and memory	0.07	0.04	2.88	0.09
	Trials previous day	0.21	0.06	13.60	< 0.001
	Trial	—	—	18.38 ^a	< 0.001
	Day*elevation	0.42	0.06	54.10	< 0.001
	Bird ID ^b	—	—		Var. = 0.22 $R^2c = 0.22$ $R^2m = 0.12$ AIC = 10412.60

SE, Standard Error. Bolded values indicate statistical significance ($\alpha < 0.05$). Conditional and marginal R^2 (R^2c and R^2m) were calculated according to Nakagawa and Schielzeth (2013). Elevation estimates relative to high elevation. Unstandardized regression coefficients used as estimates (Est.). All χ^2 statistics use 1 degree of freedom, unless otherwise indicated.

^aThe χ^2 statistic reports 3 degrees of freedom.

^bBird ID fitted as random intercept.

Appendix Figure Legends

Figure A4.1. Number of birds by elevation and age in (a) Serial R1 ($N = 97$) and (b) Serial R2 ($N = 235$). Age 0 years indicates juveniles; age 1–8 years indicates adults.

Figure A4.2. Performance in trial 1 of Serial R1 versus single reversal performance (i.e., cognitive flexibility): (a) location errors; (b) predicted probability of visiting the previous feeder. Heavy dashed line indicates expected random value.

Figure A4.3. Mean last five errors per trial per day for Serial R1. For box plots, lower and upper box boundaries represent 25th and 75th percentiles, lines inside boxes represent the median, lower and upper error lines indicate $1.5\times$ greater and lesser than the 25th and 75th percentiles, respectively. Outliers not shown. Transparent points (jittered for clearer viewing) and density plots represent raw data.

Figure A4.4. Number of neutral errors before visiting the previous feeder in the first trial per day for (a) Serial R1 ($N = 97$ birds for each day) and (b) Serial R2 ($N = 235$ birds for days 3–10 and 83 birds on day 17). The number of errors before visiting the previous feeder is a subset of the total number of errors a bird made. For example, if a bird made three total errors and two of those errors were before visiting the previous feeder, then this bird visited the previous feeder on its third visit before finding the correct feeder on its fourth visit during the trial. “NA” indicates that the bird did not visit the previous

feeder during the trial, but this does not indicate that these birds made zero errors overall. Dashed lines indicate expected random value (4.5 errors).

Figure A4.5. Mean last five errors per trial per day for Serial R2. For box plots, lower and upper box boundaries represent 25th and 75th percentiles, lines inside boxes represent the median, lower and upper error lines indicate $1.5\times$ greater and lesser than the 25th and 75th percentiles, respectively. Outliers not shown. Transparent points (jittered for clearer viewing) and density plots represent raw data.

Figure A4.6. The predicted probability of visiting the previous feeder by spatial learning and memory ability by elevation for Serial R2.

Appendix Figures

Figure A4.1

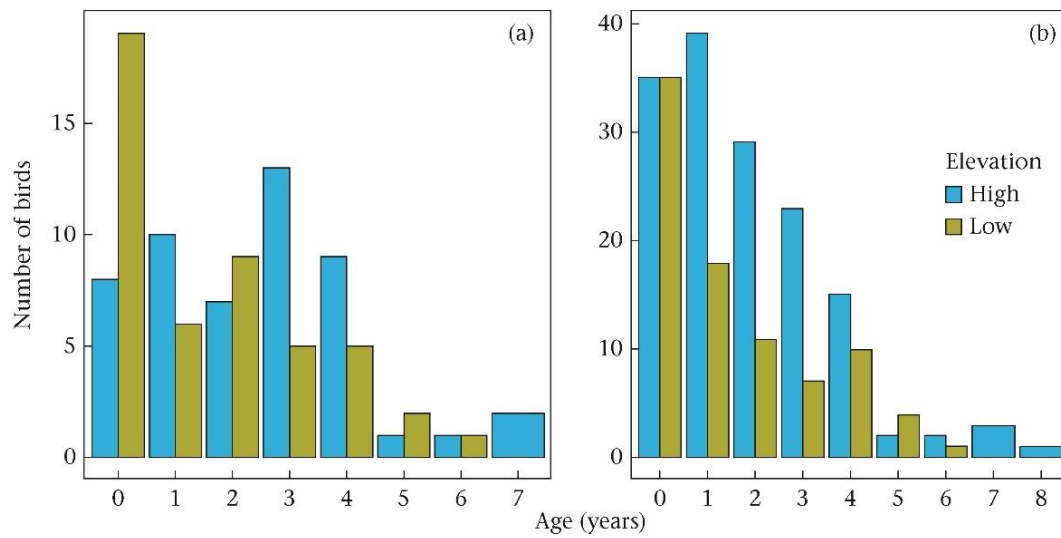


Figure A4.2

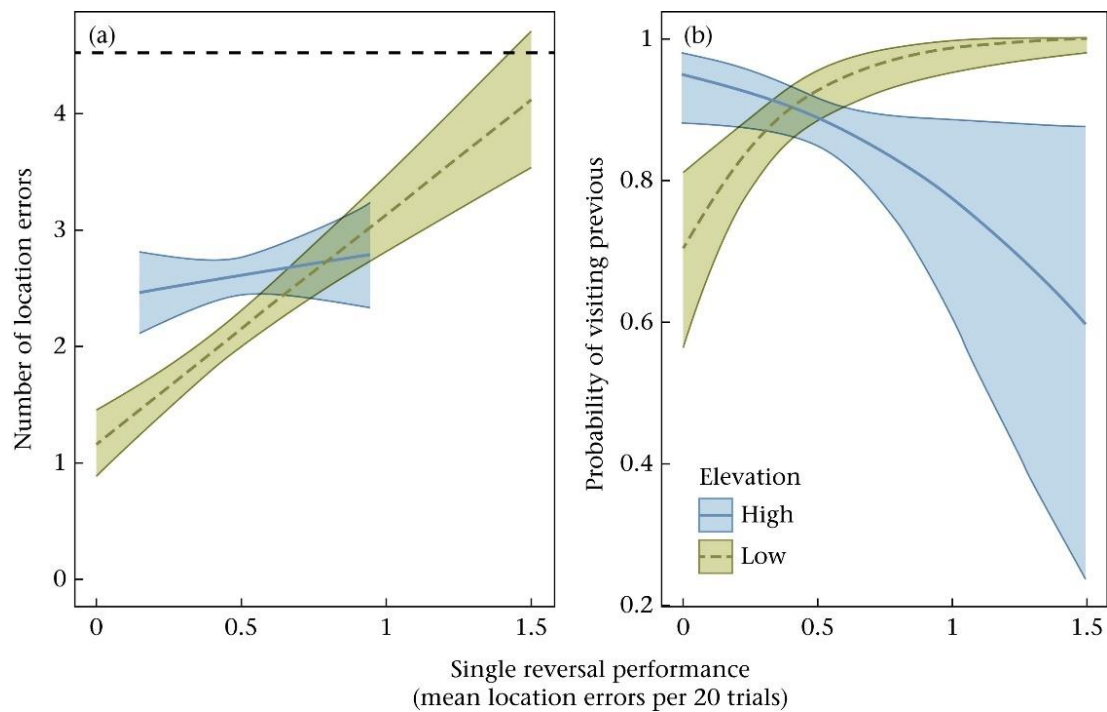


Figure A4.3

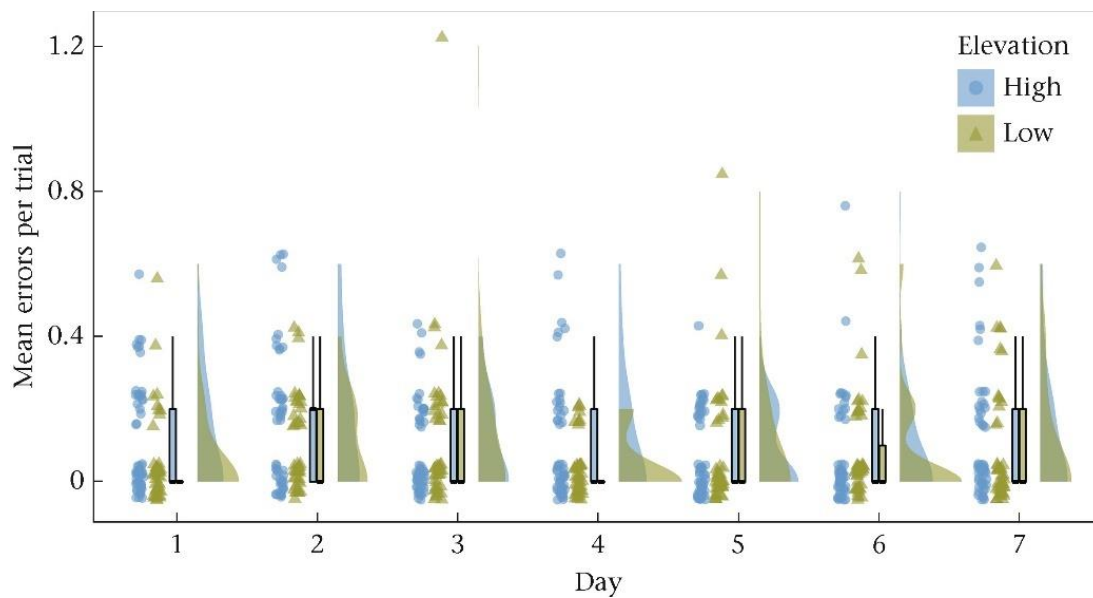


Figure A4.4

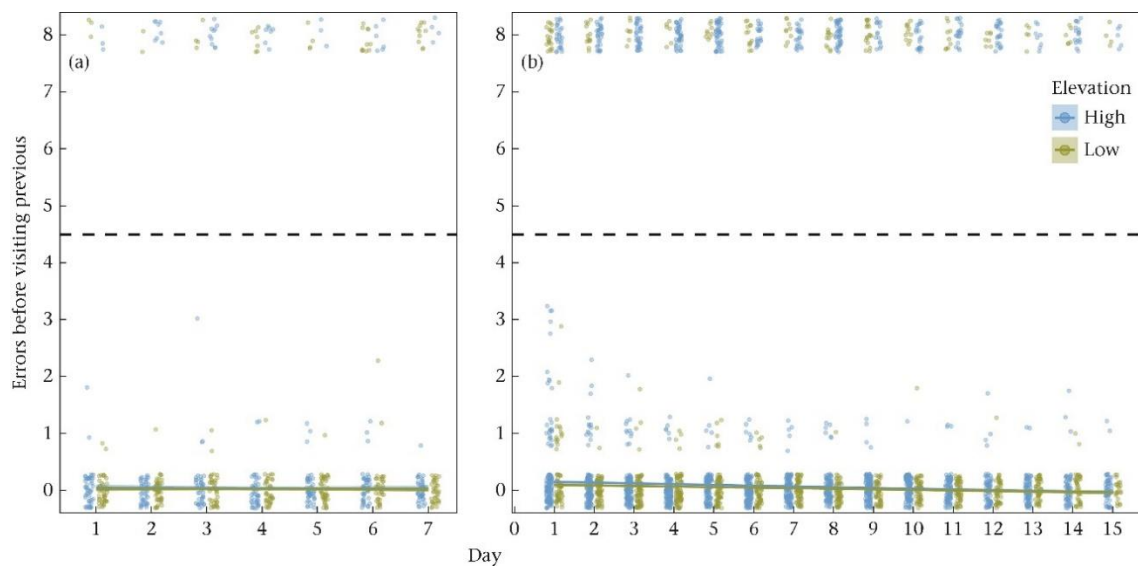


Figure A4.5

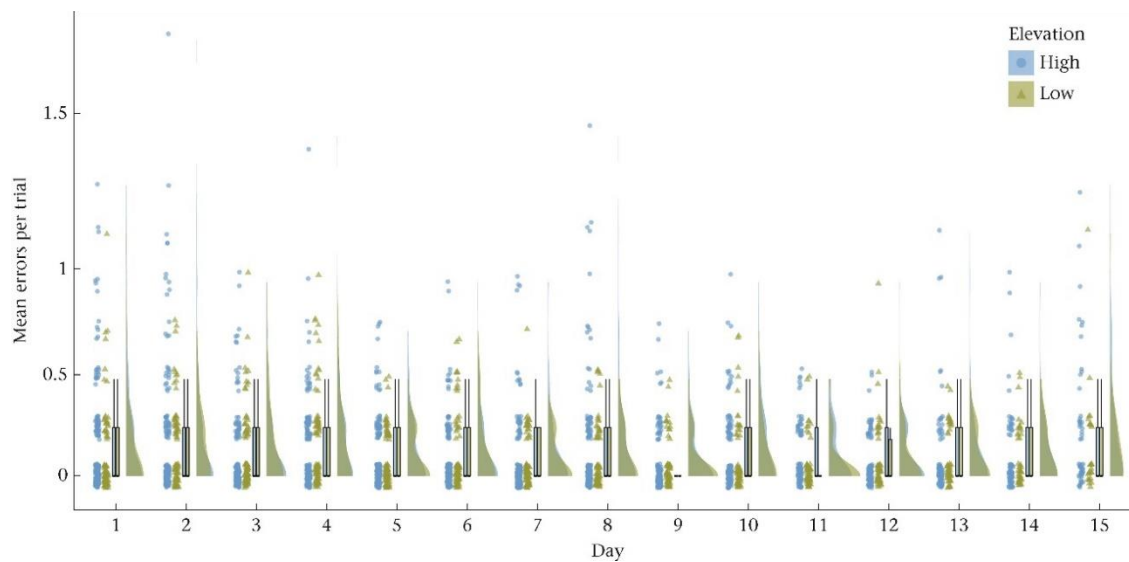
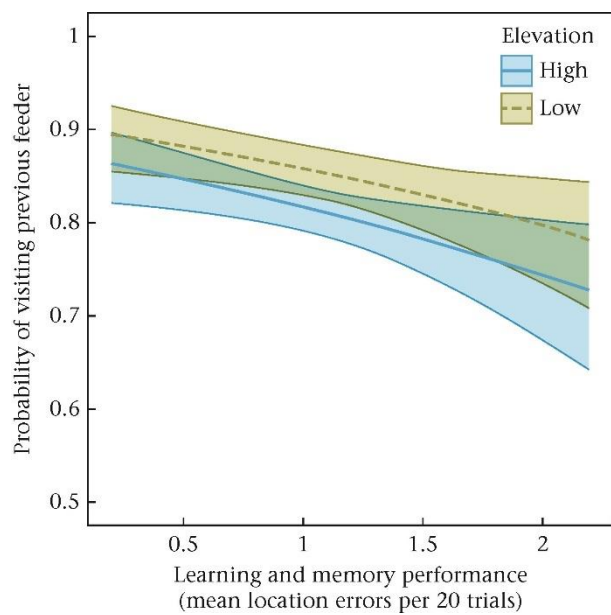


Figure A4.6



**Chapter 5 Food-Caching Mountain Chickadees Can Learn Abstract Rules to Solve
a Complex Spatial-Temporal Pattern**

Lauren M. Benedict^{1,2*}, Virginia K. Heinen¹, Joseph F. Welklin¹, Benjamin R.
Sonnenberg^{1,2}, Lauren E. Whitenack^{1,2}, Eli S. Bridge³, Vladimir V. Pravosudov^{1,2}

¹ University of Nevada Reno, Department of Biology, Reno, NV 89557, USA. ² University of Nevada
Reno, Ecology, Evolution and Conservation Biology Graduate Program, Reno, NV 89557, USA. ³
University of Oklahoma, Oklahoma Biological Survey, Norman, OK 73019, USA

*Lead contact and corresponding author, Lauren Benedict Nguyen published as L.M.
Benedict

Publication Citation

Benedict, L.M., Heinen, V.K., Welklin, J.F., Sonnenberg, B.R., Whitenack, L.E., Bridge,
E.S., Pravosudov, V.V. (2023). Food-caching mountain chickadees can learn
abstract rules to solve a complex spatial-temporal pattern. *Current Biology*, 33:1-
9. <https://doi.org/10.1016/j.cub.2023.06.036>

Abstract

The use of abstract rules in behavioral decisions is considered evidence of executive functions associated with higher-level cognition. Laboratory studies across taxa have shown that animals may be capable of learning abstract concepts, such as the relationships between items, but often use simpler cognitive abilities to solve tasks. Little is known about whether or how animals learn and use abstract rules in natural environments. Here, we tested whether wild food-caching mountain chickadees (*Poecile gambeli*) could learn an abstract rule in a spatial-temporal task in which the location of a food reward rotated daily around an 8-feeder square spatial array for up to 34 days. Chickadees initially searched for the daily food reward by visiting the most recently rewarding locations and then moving backward to visit previously rewarding feeders, using memory of previous locations. But by the end of the task chickadees were more likely to search forward in the correct direction of rotation, moving away from the previously rewarding feeders. These results suggest that chickadees learned the direction rule for daily feeder rotation and used this to guide their decisions while searching for a food reward. Thus, chickadees appear to use an executive function to make decisions on a foraging-based task in the wild.

Keywords

Avian cognition, chickadee, cognitive ecology, abstract concept, learning and memory, rule-learning, spatial cognition

Introduction

Many species have evolved cognitive strategies to learn and track where resources are located in variable environments (Dall et al., 2005; Morand-Ferron, 2017). As resources often change predictably in both time and space, animals may be able to learn and use rules to forage more efficiently (Charnov, 1976; McNamara and Houston, 1985). Rules are generalizable concepts that have been learned from previous experiences and can be transferred to new situations (Lazareva and Wasserman, 2017; Shanks and Darby, 1998), such as when to leave a resource patch to maximize foraging efficiency (Hodges, 1985; McNamara and Houston, 1985). Nonhuman animals are well-known to learn rules based on perceptual concepts, or generalization from physical characteristics (Lazareva and Wasserman, 2017). But rule-learning based on abstract concepts remains controversial (Katz and Wright, 2021), as it involves generalization based on the relationships between and among stimuli, regardless of specific physical characteristics (Katz et al., 2007; Lazareva and Wasserman, 2017). Using abstract concepts to learn and use a rule is associated with executive functions, or higher-level cognitive processes that integrate different types of information for goal oriented behaviors (Diamond, 2013). The ability to use abstract relational rules may be beneficial in variable environments with repeating conditions but has been considered supposedly unique to humans (Premack, 1983, 1978). Recently, studies have found evidence for learning abstract rules in a wide variety of diverse taxa, including non-human primates (Katz et al., 2007; Wright et al., 2003), other mammals (Herman et al., 1994; Murphy et al., 2008), birds (Hoeschele et al., 2012; Katz and Wright, 2006; Magnotti et al., 2015), fishes (Aellen et al., 2022; Miletto

Petrazzini and Brennan, 2020) and invertebrates (Avarguès-Weber et al., 2011; Weise et al., 2022). Yet in other studies, nonhuman animals often fail to fully learn or use abstract concepts (Brown and Austin, 2021; Flemming and Thompson, 2021; Fuss et al., 2021; Kroupin and Carey, 2021; Maes et al., 2015). As such, how and why abstract-learning abilities may vary across taxa or among individuals is still unclear.

Some of the controversy is due to methodologies that do not sufficiently rule out alternative strategies using simpler cognitive processes (Katz et al., 2007; Katz and Wright, 2021). For example, rule-learning has been commonly tested in laboratory conditions using a classic serial reversal task, in which individuals must repeatedly learn reward contingencies associated with two food sources (Izquierdo et al., 2017; Shettleworth, 2010). In this task, individuals could improve their performance by learning the “win-stay, lose-shift” rule: return to the rewarding cue; if it stops rewarding, switch to a different cue (Shettleworth, 2010; Strang and Sherry, 2014). This rule-based strategy likely involves executive functions to inhibit the previously learned association and shift to the correct rewarding cue (Shettleworth, 2010; Strang and Sherry, 2014; Tello-Ramos et al., 2019). But since the food reward alternates between the same two cues, an individual could simply learn to search in the order of most recent memory (Benedict et al., 2023a; Shettleworth, 2010). In other words, performance on a serial reversal learning task could demonstrate rule-learning but is often inconclusive when evaluating whether or not the learned rule was abstract.

Failure to learn an abstract rule could also be explained by alternative strategies, depending on the task design (Katz and Wright, 2006; Kroupin and Carey, 2021; Zentall,

2021). For example, a common test of abstract concept learning is the same/different task, in which individuals must learn to match or identify pairs of items as “same” or “different” and then apply this relational rule to novel items (Gentner et al., 2021). Animals fail to learn the rule if they do not categorize the new items as well as the items in the training set (Katz et al., 2007; Katz and Wright, 2021). However, this failure does not necessarily mean the animal lacks the ability to learn abstract concepts (Kroupin and Carey, 2021). If the item pairs are repeated or share physical characteristics, then individuals could potentially use simpler, perceptual concepts to learn associations with specific characteristics of the items, leading to poor performance (Katz and Wright, 2006; Maes et al., 2015). An individual might also perform poorly if it has not had previous experience using abstract rules and so has not developed the mental representations needed to learn the rule during the task (Kroupin and Carey, 2021; Smirnova et al., 2021). Similarly, without previous experience, an individual might be biased towards using certain cues, and may need to learn which cues are salient during a same/different task (Kroupin and Carey, 2021). Testing wild animals with tasks that resemble natural conditions may address these alternatives, by increasing the likelihood that individuals have already generated the necessary representations before testing. But since most studies have been conducted under laboratory conditions, it is unclear to what extent wild animals may use abstract rules in natural environments.

Here, we attempted to address these issues by using wild, food-caching mountain chickadees (*Poecile gambeli*) in their natural environment. We designed a novel experimental task to test whether chickadees could learn an abstract rule while making foraging decisions in a complex spatial-temporal task. Chickadees had to learn to track

the daily location of a food reward that moved predictably around a square spatial array of eight feeders (Figure 1). Birds could visit any feeder at the array but could only receive a food reward from one feeder per day. We used “smart” feeders (Croston et al., 2017a; Rebecca Croston et al., 2016) to reassign each bird to a new rewarding feeder every day for 34 days, always rotating the food reward to the nearest feeder in the same direction (clockwise or counterclockwise; Figure 1). Through experience with the task, birds could learn the spatial pattern (*where* the food reward is located), the temporal pattern (*when* the food reward shifts) and the abstract rule (the relative *direction* that the food reward rotates). By learning all three, birds could also learn to predict where the food reward would be located the next day.

As a food-caching species, mountain chickadees rely on spatial learning and memory to make and retrieve individually cached food items throughout the winter (Brodbeck, 1994; Pravosudov and Roth, 2013). Learning abstract rules may be advantageous because one rule could replace multiple learned associations, reducing overall cognitive load and reducing the possibility of previously learned associations interfering with the formation of new memories (i.e., proactive interference; Tello-Ramos et al., 2019). As chickadees store thousands of memories of caches (Pravosudov and Roth, 2013), any mechanism that reduces cognitive load could be highly beneficial (Tello-Ramos et al., 2019), such as forgetting unnecessary cache locations after retrieval. Moreover, chickadees appear to adjust foraging-based search behavior depending on environmental conditions (e.g., harshness) and previous experience (Benedict et al., 2021). It may be advantageous for chickadees to learn abstract rules that could be used to

predict when and where ephemeral resources will be available, especially in harsh winter conditions when caloric requirements may be high.

The design of this spatial-temporal task allowed us to discriminate between two different processes that birds could use to solve the task. The “smart” feeders recorded all visits from tagged birds, allowing us to count how many incorrect feeder locations (i.e., location errors) were visited before finding a food reward (i.e., completion of a “trial”, Table S5.1). When a bird makes a location error at the beginning of a trial, it must decide where to search for food next. If the bird remembered the previous day’s rewarding location, then it likely made this error to that same location and has now learned that the reward contingency has changed. At this point, the chickadee’s next feeder visit should provide information about which processes influenced its decision-making. First, if the chickadee relied on associative learning and memory recency, we would expect it to search for food by moving backwards to visit the locations that most recently provided food. Second, if the chickadee learned the rule of the spatial-temporal rotation, we would expect it to search forward, moving toward the correct rewarding feeder and away from the previously rewarding feeders. This second method demonstrates using an abstract rule: birds must learn that the food reward is located in the feeder one position to the left (or right) of the most recently rewarding feeder. They must then apply this learned relationship to new feeders, as the rewarding feeder location continues to rotate daily around the feeder array.

Behavior during the first trial completed each day (i.e., “trial 1”) should reflect where the bird expected to find food when first visiting the array. Unlike all subsequent

trials, trial 1 does not show evidence of learning because the bird does not learn the new rewarding feeder location until it finishes trial 1 and receives a food reward. Instead, trial 1 behavior should show how the bird searches. If chickadees learn to predict the spatial-temporal pattern using the abstract rule, they should search less during trial 1, leading to a reduction in trial 1 location errors as birds learn to go directly to the correct rewarding feeder. If birds learn to use the abstract rule, but do not learn to predict the temporal pattern, we do not expect to see perfect performance (i.e., zero trial 1 location errors) but would expect to see fewer location errors to the previous day's rewarding feeder (i.e., a reversal error) throughout the task.

Finally, to assess the role of individual cognitive ability in learning an abstract rule, we used the same spatial feeder arrays to test two spatial cognitive abilities: spatial learning and memory ability (Rebecca Croston et al., 2016; Sonnenberg et al., 2019b) and spatial reversal learning ability (Audet and Lefebvre, 2017; Tello-Ramos et al., 2019). We expected that performance on the spatial-temporal task would correlate with performance on the spatial learning and memory task if birds were using associative memory as an alternative strategy (e.g., learning each rewarding feeder location every day) but would correlate with performance on the spatial reversal learning task if birds learned to use the abstract rule. We expected that the processes that are involved in reversal learning, such as inhibiting the recall of a previous memory and simultaneously learning new, similar information (Izquierdo et al., 2017; Tello-Ramos et al., 2019), may also be associated with abstract concept learning. We do not use reversal learning performance to estimate “general intelligence” or to draw conclusions about cognitive flexibility (Audet and Lefebvre, 2017).

Results

Performance improved across trials but not across days in the spatial-temporal task

The number of location errors in trial 1 did not change significantly across days (GLMM estimate \pm SE: -0.01 ± 0.02 , $\chi_1^2 = 0.28$, $P = 0.59$) and or with the number of trials completed the previous day (0.002 ± 0.02 , $\chi_1^2 = 0.12$, $P = 0.91$; $R_{GLMM(c)}^2 = 0.13$; $R_{GLMM(m)}^2 < 0.001$; Fig. 5.2). Birds performed better than chance in trial 1 every day, suggesting that search behavior was not random (one-sample t-tests, Table S5.3). As trial 1 performance did not improve across the spatial-temporal task, birds did not appear to learn to predict the daily location of the rewarding feeder across days.

For trials 2-5, the number of location errors per trial was on average less than 1 location error and did not vary significantly across days (GLMM estimate \pm SE: -0.01 ± 0.04 , $\chi_1^2 = 0.06$, $P = 0.80$) or with the number of trials completed during the previous day (-0.03 ± 0.03 , $\chi_1^2 = 0.99$, $P = 0.32$; Fig. 5.2). Location errors per trial varied significantly among individual trials ($\chi_3^2 = 25.59$, $P < 0.001$; Fig. 5.2) and there was a significant interaction effect between trials and days since first visit ($\chi_3^2 = 8.70$, $P = 0.03$). However, differences in the rate of change among trials 2-5 were small, with mean values less than 0.5 location errors across all days (Fig. 5.2). Birds appeared to learn the location of each day's feeder rapidly after trial 1 but did not continue to improve overall performance in trials 2-5 across days.

Birds did not learn to predict the temporal component of the spatial-temporal task

Birds consistently made a reversal error (i.e., a location error to the previous day's rewarding feeder) in trial 1 throughout the spatial-temporal task. The average probability of making a reversal error in trial 1 did not change significantly with days since first visit (GLMM estimates in log-odds scale \pm SE: 0.341 ± 0.06 , $\chi^2_1 = 0.29$, $P = 0.59$), but birds that completed more trials the previous day were significantly more likely to make a reversal error in the first trial the following day (0.46 ± 0.08 , $\chi^2_1 = 30.538$, $P < 0.001$; $R^2_{GLMM(c)} = 0.109$, $R^2_{GLMM(m)} = 0.059$; $N = 55$; Fig. 5.3A). The consistency across days in making a reversal error after the food reward had moved suggests that birds did not learn the temporal component of the task.

The number of location errors made before the reversal error in trial 1 was small and increased significantly across days since first visit (GLMM negative binomial estimate \pm SE: 0.18 ± 0.05 , $\chi^2_1 = 12.29$, $P < 0.001$), but was not significantly associated with the number of trials completed the previous day (-0.09 ± 0.06 , $\chi^2_1 = 2.40$, $P = 0.12$; $R^2_{GLMM(c)} = 0.09$, $R^2_{GLMM(m)} = 0.03$, $N = 55$; Fig. 5.3B). Despite this significant increase, birds on average only made ca. 1 location error before making a reversal error, even at the end of the task. This suggests that chickadees typically started a trial by making a reversal error at the beginning of the task, but towards the end of the task more birds were instead making a location error to another feeder first.

In trials 2-5, the average probability that a bird made a reversal error also did not vary significantly across days (GLMM estimate in log-odds scale \pm SE: -0.11 ± 0.07 , $\chi^2_1 = 2.22$, $P = 0.14$), among trials ($\chi^2_3 = 3.50$, $P = 0.32$), with the number of trials completed

the previous day (-0.01 ± 0.05 , $\chi_1^2 = 0.02$, $P = 0.88$), nor with an interaction between days since first visit and trials ($\chi_3^2 = 3.88$, $P = 0.27$; $R_{GLMM(c)}^2 = 0.05$, $R_{GLMM(m)}^2 = 0.004$; $N = 55$; Fig. 5.3A). Unlike in trial 1, birds were unlikely to make a reversal error in trials 2-5 throughout the whole spatial-temporal task.

Birds learned the abstract direction rule across days in the spatial-temporal task

The probability that a bird moved toward the correct feeder (i.e., in the direction of feeder rotation) after making a reversal error in trial 1 of each day significantly increased across days since the bird's first visit (GLMM estimate in log-odds scale \pm SE: 0.30 ± 0.06 , $\chi_1^2 = 24.55$, $P < 0.001$), but was not significantly associated with the number of trials completed the previous day (-0.02 ± 0.07 , $\chi_1^2 = 0.08$, $P = 0.78$; $R_{GLMM(c)}^2 = 0.10$, $R_{GLMM(m)}^2 = 0.03$; $N = 55$; Fig. 5.4A). At the same time, the probability that a bird moved backwards after making a reversal error (i.e., away from the correct rewarding feeder) significantly decreased across days since the bird's first visit (GLMM estimate in log-odds scale \pm SE: -0.28 ± 0.06 , $\chi_1^2 = 20.80$, $P < 0.001$; previous day trials: -0.05 ± 0.07 , $\chi_1^2 = 0.52$, $P = 0.47$, $R_{GLMM(c)}^2 = 0.08$, $R_{GLMM(m)}^2 = 0.02$; $N = 55$; Fig. 5.4B). Birds rarely moved across the array after visiting the previously rewarding feeder (4% of 1242 trials). Overall, as the task continued, birds were less likely to move backwards to the feeders that were rewarding most recently and were more likely to move forward toward the new rewarding feeder.

To address the possibility that birds could be biased to continue moving in a consistent direction around the arrays, we repeated this direction analysis with a conservative subset of only trials that started with a reversal error (825 trials, N = 55 unique birds). This subset excluded 34% of trials in the previous analysis and yielded similar results: the probability of moving toward the correct rewarding feeder increased from below random chance (50%) to above random chance by the end of the task (GLMM estimate in log-odds scale \pm SE: 0.21 ± 0.07 , $\chi_1^2 = 8.10$, $P = 0.004$; previous day trials: 0.13 ± 0.08 , $\chi_1^2 = 2.54$, $P = 0.11$; $R_{GLMM(c)}^2 = 0.09$, $R_{GLMM(m)}^2 = 0.02$; N = 55; Fig. 5.4a). The probability to move backwards from the previous feeder also decreased significantly throughout the task (GLMM estimate in log-odds scale \pm SE: -0.21 ± 0.07 , $\chi_1^2 = 8.10$, $P = 0.004$; trials completed the previous day: -0.20 ± 0.08 , $\chi_1^2 = 6.09$, $P = 0.01$; $R_{GLMM(c)}^2 = 0.09$, $R_{GLMM(m)}^2 = 0.02$; N = 55; Fig. 5.4b). Birds rarely moved to the opposite side of the feeder array after visiting the previous feeder (3% of 825 trials). Overall, these results were consistent with previous models: as the task progressed, birds were less likely to move backwards to visit recently rewarding feeders and were more likely to move forward toward the correct feeder, after making a reversal error to the previous day's rewarding feeder.

No effect of spatial learning and memory performance on performance in spatial-temporal task

The number of location errors in trial 1 each day was not significantly associated with spatial learning and memory performance (GLMM estimate \pm SE: -0.11 ± 0.10 , χ_1^2

= 1.10, $P = 0.29$; days since first visit: -0.02 ± 0.06 , $\chi_1^2 = 0.11$, $P = 0.74$; $R_{GLMM(c)}^2 = 0.08$; $R_{GLMM(m)}^2 = 0.002$; $N = 49$). The average probability that a bird made a reversal error in trial 1 was also not significantly associated with spatial learning and memory performance (GLMM estimates in log-odds scale \pm SE: 0.01 ± 0.09 , $\chi_1^2 = 0.02$, $P = 0.90$; $R_{GLMM(c)}^2 = 0.10$, $R_{GLMM(m)}^2 = 0.05$; $N = 49$). The direction birds moved after making a reversal error did not vary with spatial learning and memory for the conservative dataset, either for the probability of moving toward the correct feeder location (GLMM estimate in log-odds scale \pm SE: 0.07 ± 0.08 , $\chi_1^2 = 0.40$, $P = 0.53$; $R_{GLMM(c)}^2 = 0.08$, $R_{GLMM(m)}^2 = 0.01$; $N = 49$;) or away from the correct feeder location (GLMM estimate in log-odds scale \pm SE: -0.01 ± 0.10 , $\chi_1^2 = 0.01$, $P = 0.93$; $R_{GLMM(c)}^2 = 0.08$, $R_{GLMM(m)}^2 = 0.02$; $N = 49$).

No effect of spatial reversal learning performance on performance in spatial-temporal task

The number of location errors in trial 1 each day was also not significantly associated with reversal learning performance (GLMM estimate \pm SE: 0.03 ± 0.11 , $\chi_1^2 = 0.09$, $P = 0.77$; days since first visit: $-0.0247 \pm$ SE = 0.06 , $\chi_1^2 = 0.19$, $P = 0.66$; $R_{GLMM(c)}^2 = 0.08$, $R_{GLMM(m)}^2 < 0.001$; $N = 47$). The average probability that a bird made a reversal error in trial 1 was not significantly associated with reversal learning performance (GLMM estimates in log-odds scale \pm SE: -0.06 ± 0.09 , $\chi_1^2 = 0.49$, $P = 0.48$; $R_{GLMM(c)}^2 = 0.09$, $R_{GLMM(m)}^2 = 0.04$; $N = 47$).

Birds made almost no location errors in the last 5 trials completed per day

Birds successfully learned the daily rewarding location by the end of each day: every day, birds made fewer than 0.5 mean location errors per trial in the last 5 trials of each day. The sum of location errors in the last 5 trials per day significantly decreased across days (GLMM estimate \pm SE: -0.14 ± 0.03 , $\chi_1^2 = 17.57$, $P < 0.001$; $R_{GLMM(c)}^2 = 0.13$, $R_{GLMM(m)}^2 = 0.02$; $N = 55$), but the number of errors was close to zero across the entire task (Figure S2).

Discussion

Overall, our data showed that wild, food-caching chickadees were able to learn an abstract rule for a complex spatial-temporal pattern in a natural setting. To our knowledge, this is the first time that abstract concept learning has been demonstrated outside of a laboratory setting without using a serial reversal task. As predicted, as birds learned the spatial-temporal task, they appeared to rely less on spatial learning and memory and more on the direction rule to decide where to search next. Although at the beginning, chickadees were less likely than chance to search in the correct direction of rotation (and equally likely to search away from the correct feeder), by the end of the task chickadees were significantly more likely to search toward the correct rewarding feeder (and less likely to search away). Due to the setup of the task, the choices were mutually exclusive: a bird could either use memory recency to move backwards, searching feeders it likely remembered from previous days, or it could search toward the correct feeder, likely using the direction rule it had learned during the task. It is unlikely that birds could use memory recency or associative learning alone, without a mental representation of direction, to achieve this better-than-chance performance. Moreover, chickadees in this study system do not show directional bias while searching the feeder arrays for a food reward (Benedict et al., 2023b), suggesting that the significant tendency to move in the direction of rotation is not likely due to passive directional biases or laterality in movement.

Although birds appeared to learn the directional rule, they did not learn to predict the temporal part of the task. Chickadees learned to search in the correct direction despite

no significant change in trial 1 performance (i.e., the number of location errors or the likelihood of making a reversal error). Moreover, there was no significant association between these trial 1 metrics with either spatial learning and memory ability or spatial reversal learning performance. While the former result supports our prediction that chickadees did not use simple associative learning to solve the spatial-temporal task, the latter was unexpected. One possible explanation is that chickadees at high elevation may rely strongly on spatial learning and memory to survive harsh winter environments (Croston et al., 2017a; Rebecca Croston et al., 2016; Kozlovsky et al., 2018b; Pitera et al., 2018) and as such their reversal learning ability may be constrained by strong associations associated with winter caching. We have previously found that chickadees at lower, milder elevations performed better on tasks of reversal learning than chickadees at the higher elevation sites tested in this study (Benedict et al., 2023a; Tello-Ramos et al., 2018). Additionally, these low elevation birds were capable of learning to predict the location of a food reward across days in a serial reversal task, unlike high elevation birds (Benedict et al., 2023a), suggesting there may be constraints to flexibility in a spatial context for the birds in this study. Unfortunately, due to low participation, we only analyzed one bird from low elevation and could not test for elevational differences in the present study. We speculate that motivation for low elevation birds to participate may have been low due to abundant natural food sources (i.e., pine nuts).

Evidence for abstract-concept learning

Learning the direction rule presents evidence for abstract rule-based learning in wild mountain chickadees. The concept of direction in this task likely differs from other uses of direction in navigation. For example, desert ants navigate using path integration, in which individuals use the speed, distance and direction previously traveled to update their position relative to a starting position (Heinze et al., 2018). But the representation of direction in path integration may not be abstract because it depends on specific characteristics of the landscape and the previous path that the animal traveled along. In contrast, birds in this study needed to learn the relative direction between previous rewarding feeder locations and then generalize this to new instances (days). By rotating the feeders every day, we prevented birds from learning the rule through simple associations. If a bird associated a given feeder's position with food one day, that bird could not use the same association to find food the next day. Considering that birds did not predict the temporal pattern, they may have learned the rule to search for food in the same direction of rotation. This abstract rule could be summarized as “win-stay, lose-shift-forward”, to make a comparison with the optimal strategy to solve a serial reversal task, the “win-stay, lose-shift” rule. Indeed, there was no correlation between individual spatial learning and memory performance and trial 1 performance on the spatial-temporal task, suggesting that search behavior was not likely determined primarily by associative learning ability. Finally, birds need to inhibit previous memories to learn to move forward, and such inhibition is also part of executive control (Veit and Nieder, 2013).

Abstract-concept learning associated with the “same vs different” concept has been shown in many bird species, including pigeons (Bobrowicz and Greiff, 2022; Katz and Wright, 2006), corvids (Bobrowicz and Greiff, 2022; Magnotti et al., 2017, 2015; Veit and Nieder, 2013) and closely related black-capped chickadees, *Poecile atricapillus* (Hoeschele et al., 2012). But reported performance varies widely, and the set sizes needed to train individuals to learn the abstract rules vary from hundreds to thousands (Katz et al., 2007). Other types of rule-learning have been documented in birds as well, including ordering rules to recognize auditory sequences (Suzuki et al., 2017) and other rules that resemble language or grammar (van Heijningen et al., 2013). There has been debate about whether apparent abstract-concept learning could be better explained by other cognitive processes, such as associative learning or feature-based generalization (Katz and Wright, 2021, 2006; Maes et al., 2015). A key difficulty in traditional abstract concept-learning tasks is the amount of featural overlap between the learned and transfer stimuli; when the tested stimuli share characteristics with the transfer stimuli, animals may be able to use simple, alternative processes to solve the task instead of a rule (Katz et al., 2007). Our spatial-temporal task design limits featural overlap because the feeders were virtually identical in every dimension except for spatial location. The feeder construction ensured that the shape, size, color, material and reward type for each feeder were the same, and even minor differences in appearance could not be used to find the correct food reward. Moreover, food-caching birds primarily use spatial cues over local cues (Brodbeck, 1994; Clayton and Krebs, 1994; Rebecca Croston et al., 2016), making it less likely that featural overlap could have led birds to learn the rule through simple associative learning. Our spatial-temporal task also differed from previous abstract

learning studies because it was conducted with wild, free-living birds in their natural habitat using a foraging task that allowed birds to move freely. Thus, the decisions about where to search during this task likely resembled decisions made during typical foraging behavior. It is well known that chickadees use specialized spatial cognitive abilities to retrieve self-made food caches during the fall and winter; but it has been less studied whether chickadees also use spatial cognition to make foraging decisions. Our spatial-temporal task likely resembles foraging from food sources that vary in accessibility due to harsh storms that prevent access to foraging during the winter. Although spatial tasks have been underutilized for studying abstract-concept learning, such tasks may provide unique advantages.

Conclusions

Our study presents evidence for abstract rule-learning in wild birds in their natural environments, using a complex spatial-temporal pattern. We conclude that the behavior observed in this foraging task could only be explained by abstract rule-learning, as birds could only learn to search in the correct direction based on transferring learned associations to new feeders in the array. Yet birds in this study did not learn to predict the spatial-temporal pattern, suggesting that there may be limits to the types of temporal cues or the range of abstraction that is possible for these birds. Using abstract rules could be adaptive in harsh or variable conditions, allowing animals to rapidly adjust their behavior to forage more efficiently. As there was substantial individual variation in abstract-learning abilities, the next question is whether natural selection could potentially act on

this individual variation in chickadees. Future directions should aim to understand the mechanisms driving this variation, and whether it may be associated with heritable, genetic variation or may arise from individual differences in previous experience. We think it is important to conduct tasks that are ecologically relevant to explore not only whether nonhuman animals can demonstrate executive functions, but also whether animals use these higher-level abilities for decision-making with real consequences in the wild.

Acknowledgements

Thank you to the staff of Sagehen Experimental Forest and Sagehen Creek Field Station (University of California, Berkeley) for assistance. This work was supported by the National Science Foundation (NSF) IOS1856181 and IOS2119824 to VVP. LMB and BRS were supported by the NSF Graduate Research Fellowship Program under Grant No. 2020305313 and 2019287870. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. Critical and constructive comments from four anonymous reviewers significantly improved the manuscript.

Author Contributions

Conceptualization, L.M.B and V.V.P.; Methodology, L.M.B., V.K.H., E.S.B., and V.V.P., Investigation, L.M.B., V.K.H., J.F.W., B.R.S., L.E.W., and V.V.P.; Data

curation, V.K.H. and B.R.S.; Formal analysis, L.M.B.; Writing – Original draft preparation, L.M.B. and V.V.P.; Visualization, L.M.B.; Writing – Reviewing and editing, L.M.B., V.K.H., J.F.W., B.R.S., L.E.W., E.S.B., and V.V.P.; Funding acquisition, L.M.B., B.R.S., and V.V.P.; Project administration, V.V.P., Supervision, V.V.P.

Inclusion and Diversity

We support inclusive, diverse, and equitable conduct of research.

Declaration of Interests

The authors declare no competing interests.

References

- Aellen, M., Siebeck, U. E., & Bshary, R. (2022). Cleaner wrasse *Labroides dimidiatus* perform above chance in a “matching-to-sample” experiment. *PLOS ONE*, 17(1), e0262351. <https://doi.org/10.1371/journal.pone.0262351>
- Audet, J.-N., & Lefebvre, L. (2017). What’s flexible in behavioral flexibility? *Behavioral Ecology*, 28(4), 943–947. <https://doi.org/10.1093/beheco/axx007>
- Avarguès-Weber, A., Dyer, A. G., & Giurfa, M. (2011). Conceptualization of above and below relationships by an insect. *Proceedings of the Royal Society B: Biological Sciences*, 278(1707), 898–905. <https://doi.org/10.1098/rspb.2010.1891>
- Bates, D., Machler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Bridge, E. S., & Pravosudov, V. V. (2023). Learning predictably changing spatial patterns across days in a food-caching bird. *Animal Behaviour*, 196, 55–81. <https://doi.org/10.1016/j.anbehav.2022.11.005>
- Benedict, L. M., Heinen, V. K., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., & Pravosudov, V. V. (2023). Food-caching chickadees do not exhibit directional bias when learning a spatial task. *Behavioral Ecology and Sociobiology*, 77(1), 4. <https://doi.org/10.1007/s00265-022-03275-6>
- Benedict, L. M., Pitera, A. M., Branch, C. L., Sonnenberg, B. R., Heinen, V. K., Bridge, E. S., & Pravosudov, V. V. (2021). Information maintenance of food sources is associated with environment, spatial cognition and age in a food-caching bird. *Animal Behaviour*, 182, 153–172. <https://doi.org/10.1016/j.anbehav.2021.10.009>
- Bobrowicz, K., & Greiff, S. (2022). Executive functions in birds. *Birds*, 3(2), 184–220. <https://doi.org/10.3390/birds3020013>
- Branch, C. L., Semenov, G. A., Wagner, D. N., Sonnenberg, B. R., Pitera, A. M., Bridge, E. S., Taylor, S. A., & Pravosudov, V. V. (2022). The genetic basis of spatial cognitive variation in a food-caching bird. *Current Biology*, 32(1), 210–219.e4. <https://doi.org/10.1016/j.cub.2021.10.036>
- Bridge, E. S., Wilhelm, J., Pandit, M., Moreno, A., Curry, C., Pearson, T., Proppe, D., Holwerda, C., Eadie, J., Stair, T., Olson, A., Lyon, B., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Sonnenberg, B. R., Pravosudov, V. V., & Ruyle, J. (2019). An Arduino-based RFID platform for animal research. *Frontiers in Ecology and Evolution*, 7, 257. <https://doi.org/10.3389/fevo.2019.00257>

- Brodbeck, D. R. (1994). Memory for spatial and local cues: A comparison of a storing and a nonstoring species. *Animal Learning & Behavior*, 22(2), 119–133. <https://doi.org/10.3758/BF03199912>
- Brown, M. F., & Austin, B. P. (2021). Bees and abstract concepts. *Current Opinion in Behavioral Sciences*, 37, 140–145. <https://doi.org/10.1016/j.cobeha.2020.12.002>
- Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, 9(2), 129–136. [https://doi.org/10.1016/0040-5809\(76\)90040-X](https://doi.org/10.1016/0040-5809(76)90040-X)
- Clayton, N. S., & Krebs, J. R. (1994). Memory for spatial and object-specific cues in food-storing and non-storing birds. *Journal of Comparative Physiology A*, 174(3). <https://doi.org/10.1007/BF00240218>
- Croston, R., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Bridge, E. S., Parchman, T., & Pravosudov, V. V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Animal Behaviour*, 123, 139–149. <https://doi.org/10.1016/j.anbehav.2016.10.004>
- Croston, R., Kozlovsky, D. Y., Branch, C. L., Parchman, T. L., Bridge, E. S., & Pravosudov, V. V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Animal Behaviour*, 111, 225–234. <https://doi.org/10.1016/j.anbehav.2015.10.015>
- Dall, S. R. X., Giraldeau, L.-A., Olsson, O., McNamara, J. M., & Stephens, D. W. (2005). Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, 20(4), 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>
- Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, 64(1), 135–168. <https://doi.org/10.1146/annurev-psych-113011-143750>
- Flemming, T. M., & Thompson, R. K. (2021). Same-different conceptualization: Evolutionary, developmental and neurocognitive implications for behavioral science. *Current Opinion in Behavioral Sciences*, 37, 153–157. <https://doi.org/10.1016/j.cobeha.2020.12.006>
- Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (3rd ed.). Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Fuss, T., John, L., & Schluessel, V. (2021). Same or different? Abstract relational concept use in juvenile bamboo sharks and Malawi cichlids. *Current Zoology*, 67(3), 279–292. <https://doi.org/10.1093/cz/zoy059>
- Gentner, D., Shao, R., Simms, N., & Hespos, S. (2021). Learning same and different relations: Cross-species comparisons. *Current Opinion in Behavioral Sciences*, 37, 84–89. <https://doi.org/10.1016/j.cobeha.2020.11.013>

- Hartig, F. (2021). DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models (0.4.1) [Computer software]. <https://CRAN.R-project.org/package=DHARMA>
- Heinen, V. K., Benedict, L. M., Sonnenberg, B. R., Bridge, E. S., Farine, D. R., & Pravosudov, V. V. (2022). Experimental manipulation of food distribution alters social networks and information transmission across environments in a food-caching bird. *Animal Behaviour*, 193, 1–12. <https://doi.org/10.1016/j.anbehav.2022.08.007>
- Heinen, V. K., Pitera, A. M., Sonnenberg, B. R., Benedict, L. M., Bridge, E. S., Farine, D. R., & Pravosudov, V. V. (2021). Food discovery is associated with different reliance on social learning and lower cognitive flexibility across environments in a food-caching bird. *Proceedings of the Royal Society B: Biological Sciences*, 288, 20202843. <https://doi.org/10.1098/rspb.2020.2843>
- Heinze, S., Narendra, A., & Cheung, A. (2018). Principles of insect path integration. *Current Biology*, 28(17), R1043–R1058. <https://doi.org/10.1016/j.cub.2018.04.058>
- Herman, L. M., Pack, A. A., & Wood, A. M. (1994). Bottlenose dolphins can generalize rules and develop abstract concepts. *Marine Mammal Science*, 10(1), 70–80. <https://doi.org/10.1111/j.1748-7692.1994.tb00390.x>
- Hodges, C. M. (1985). Bumble bee foraging: The threshold departure rule. *Ecology*, 66(1), 179–187. <https://doi.org/10.2307/1941318>
- Hoeschele, M., Cook, R. G., Guillette, L. M., Hahn, A. H., & Sturdy, C. B. (2012). Auditory same/different concept learning and generalization in Black-Capped Chickadees (*Poecile atricapillus*). *PLoS ONE*, 7(10), e47691. <https://doi.org/10.1371/journal.pone.0047691>
- Izquierdo, A., Brigman, J. L., Radke, A. K., Rudebeck, P. H., & Holmes, A. (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience*, 345, 12–26. <https://doi.org/10.1016/j.neuroscience.2016.03.021>
- Katz, J. S., & Wright, A. A. (2006). Same/different abstract-concept learning by pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, 32(1), 80–86. <https://doi.org/10.1037/0097-7403.32.1.80>
- Katz, J. S., & Wright, A. A. (2021). Issues in the comparative cognition of same/different abstract-concept learning. *Current Opinion in Behavioral Sciences*, 37, 29–34. <https://doi.org/10.1016/j.cobeha.2020.06.009>

- Katz, J. S., Wright, A. A., & Bodily, K. D. (2007). Issues in the comparative cognition of abstract-concept learning. *Comparative Cognition & Behavior Reviews*, 2, 79–92. <https://doi.org/10.3819/ccbr.2008.20005>
- Kozlovsky, D. Y., Branch, C. L., Pitera, A. M., & Pravosudov, V. V. (2018). Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *Royal Society Open Science*, 5, 171604. <https://doi.org/10.1098/rsos.181248>
- Kroupin, I., & Carey, S. (2021). Population differences in performance on Relational Match to Sample (RMTS) sometimes reflect differences in inductive biases alone. *Current Opinion in Behavioral Sciences*, 37, 75–83. <https://doi.org/10.1016/j.cobeha.2020.11.006>
- Lazareva, O. F., & Wasserman, E. A. (2017). Categories and Concepts in Animals. In *Learning and Memory: A Comprehensive Reference* (pp. 111–139). Elsevier. <https://doi.org/10.1016/B978-0-12-809324-5.21008-0>
- Lenth, R. (2020). emmeans: Estimated marginal means, aka least-squares means (1.4.7) [Computer software]. <https://CRAN.R-project.org/package=emmeans>
- Ludecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models [Computer software]. 10.21105/joss.00772 1
- Ludecke, D., Makowski, D., & Waggoner, P. (2020). performance: Assessment of regression models performance (0.4.4) [Computer software]. <https://CRAN.R-project.org/package=performance>
- Maes, E., De Filippo, G., Inkster, A. B., Lea, S. E. G., De Houwer, J., D’Hooge, R., Beckers, T., & Wills, A. J. (2015). Feature- versus rule-based generalization in rats, pigeons and humans. *Animal Cognition*, 18(6), 1267–1284. <https://doi.org/10.1007/s10071-015-0895-8>
- Magnotti, J. F., Katz, J. S., Wright, A. A., & Kelly, D. M. (2015). Superior abstract-concept learning by Clark’s nutcrackers (*Nucifraga columbiana*). *Biology Letters*, 11(5), 20150148. <https://doi.org/10.1098/rsbl.2015.0148>
- Magnotti, J. F., Wright, A. A., Leonard, K., Katz, J. S., & Kelly, D. M. (2017). Abstract-concept learning in Black-billed magpies (*Pica hudsonia*). *Psychonomic Bulletin & Review*, 24(2), 431–435. <https://doi.org/10.3758/s13423-016-1132-4>
- McNamara, J. M., & Houston, A. I. (1985). Optimal foraging and learning. *Journal of Theoretical Biology*, 117(2), 231–249. [https://doi.org/10.1016/S0022-5193\(85\)80219-8](https://doi.org/10.1016/S0022-5193(85)80219-8)

- Miletto Petrazzini, M. E., & Brennan, C. H. (2020). Application of an abstract concept across magnitude dimensions by fish. *Scientific Reports*, 10(1), 16935. <https://doi.org/10.1038/s41598-020-74037-5>
- Morand-Ferron, J. (2017). Why learn? The adaptive value of associative learning in wild populations. *Current Opinion in Behavioral Sciences*, 16, 73–79. <https://doi.org/10.1016/j.cobeha.2017.03.008>
- Murphy, R. A., Mondragon, E., & Murphy, V. A. (2008). Rule learning by rats. *Science*, 319, 1849–1851. <https://doi.org/10.1126/science.1151564>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Pitera, A. M., Branch, C. L., Bridge, E. S., & Pravosudov, V. V. (2018). Daily foraging routines in food-caching mountain chickadees are associated with variation in environmental harshness. *Animal Behaviour*, 143, 93–104. <https://doi.org/10.1016/j.anbehav.2018.07.011>
- Pravosudov, V. V. (1985). Search for and storage of food by *Parus cinctus lapponicus* and *P. borealis* (Paridae). *Zoologicheskii Zhurnal*, 64(7), 1036–1043.
- Pravosudov, V. V. (2006). On seasonality in food-storing behaviour in parids: Do we know the whole story? *Animal Behaviour*, 71(6), 1455–1460. <https://doi.org/10.1016/j.anbehav.2006.01.006>
- Pravosudov, V. V., & Roth, T. C., II. (2013). Cognitive ecology of food hoarding: The evolution of spatial memory and the hippocampus. *Annual Review of Ecology, Evolution, and Systematics*, 44, 173–193. <https://doi.org/10.1146/annurev-ecolsys-110512-135904>
- Premack, D. (1978). On the abstractness of human concepts: Why it would be difficult to talk to a pigeon. In S. H. Hulse, H. Fowler, & W. K. Honig (Eds.), *Cognitive Processes in Animal Behavior* (1st ed., pp. 423–451). Erlbaum.
- Premack, D. (1983). Animal Cognition. *Annual Review of Psychology*, 34(1), 351–362. <https://doi.org/10.1146/annurev.ps.34.020183.002031>
- R Core Team. (2021). R: a language and environment for statistical computing (4.1.0) [Computer software]. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Shanks, D. R., & Darby, R. J. (1998). Feature- and rule-based generalization in human associative learning. *Journal of Experimental Psychology: Animal Behavior Processes*, 24(4), 405–415. <https://doi.org/10.1037/0097-7403.24.4.405>

- Shettleworth, S. J. (2010). *Cognition, Evolution, and Behavior* (2nd ed.). Oxford University Press.
- Smirnova, A. A., Obozova, T. A., Zorina, Z. A., & Wasserman, E. A. (2021). How do crows and parrots come to spontaneously perceive relations-between-relations? *Current Opinion in Behavioral Sciences*, 37, 109–117. <https://doi.org/10.1016/j.cobeha.2020.11.009>
- Sonnenberg, B. R., Branch, C. L., Pitera, A. M., Bridge, E., & Pravosudov, V. V. (2019a). Natural Selection and Spatial Cognition in Wild Food-Caching Mountain Chickadees. *Current Biology*, 29(4), 670–676.e3. <https://doi.org/10.1016/j.cub.2019.01.006>
- Sonnenberg, B. R., Branch, C. L., Pitera, A. M., Bridge, E. S., & Pravosudov, V. V. (2019b). Natural selection and spatial cognition in wild food-caching mountain chickadees. *Current Biology*, 29(4), 670–676. <https://doi.org/10.1016/j.cub.2019.01.006>
- Strang, C. G., & Sherry, D. F. (2014). Serial reversal learning in bumblebees (*Bombus impatiens*). *Animal Cognition*, 17(3), 723–734. <https://doi.org/10.1007/s10071-013-0704-1>
- Suzuki, T. N., Wheatcroft, D., & Griesser, M. (2017). Wild birds use an ordering rule to decode novel call sequences. *Current Biology*, 27(15), 2331–2336.e3. <https://doi.org/10.1016/j.cub.2017.06.031>
- Tello-Ramos, M. C., Branch, C. L., Kozlovsky, D. Y., Pitera, A. M., & Pravosudov, V. V. (2019). Spatial memory and cognitive flexibility trade-offs: To be or not to be flexible, that is the question. *Animal Behaviour*, 129–136. <https://doi.org/10.1016/j.anbehav.2018.02.019>
- Tello-Ramos, M. C., Branch, C. L., Pitera, A. M., Kozlovsky, D. Y., Bridge, E. S., & Pravosudov, V. V. (2018). Memory in wild mountain chickadees from different elevations: Comparing first-year birds with older survivors. *Animal Behaviour*, 137, 149–160. <https://doi.org/10.1016/j.anbehav.2017.12.019>
- van Heijningen, C. A. A., Chen, J., van Laatum, I., van der Hulst, B., & ten Cate, C. (2013). Rule learning by zebra finches in an artificial grammar learning task: Which rule? *Animal Cognition*, 16(2), 165–175. <https://doi.org/10.1007/s10071-012-0559-x>
- Veit, L., & Nieder, A. (2013). Abstract rule neurons in the endbrain support intelligent behaviour in corvid songbirds. *Nature Communications*, 4(1), 2878. <https://doi.org/10.1038/ncomms3878>

- Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4th ed.). Springer. <https://www.stats.ox.ac.uk/pub/MASS4/>
- Weise, C., Ortiz, C. C., & Tibbetts, E. A. (2022). Paper wasps form abstract concept of “same and different.” *Proceedings of the Royal Society B: Biological Sciences*, 289(1979), 20221156. <https://doi.org/10.1098/rspb.2022.1156>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* [Computer software]. Springer-Verlag.
- Wright, A. A., Rivera, J. J., Katz, J. S., & Bachevalier, J. (2003). Abstract-concept learning and list-memory processing by capuchin and rhesus monkeys. *Journal of Experimental Psychology: Animal Behavior Processes*, 29(3), 184–198. <https://doi.org/10.1037/0097-7403.29.3.184>
- Zentall, T. R. (2021). Sameness may be a natural concept that does not require learning. *Current Opinion in Behavioral Sciences*, 37, 7–12. <https://doi.org/10.1016/j.cobeha.2020.05.009>

Figures

Figure 5.1

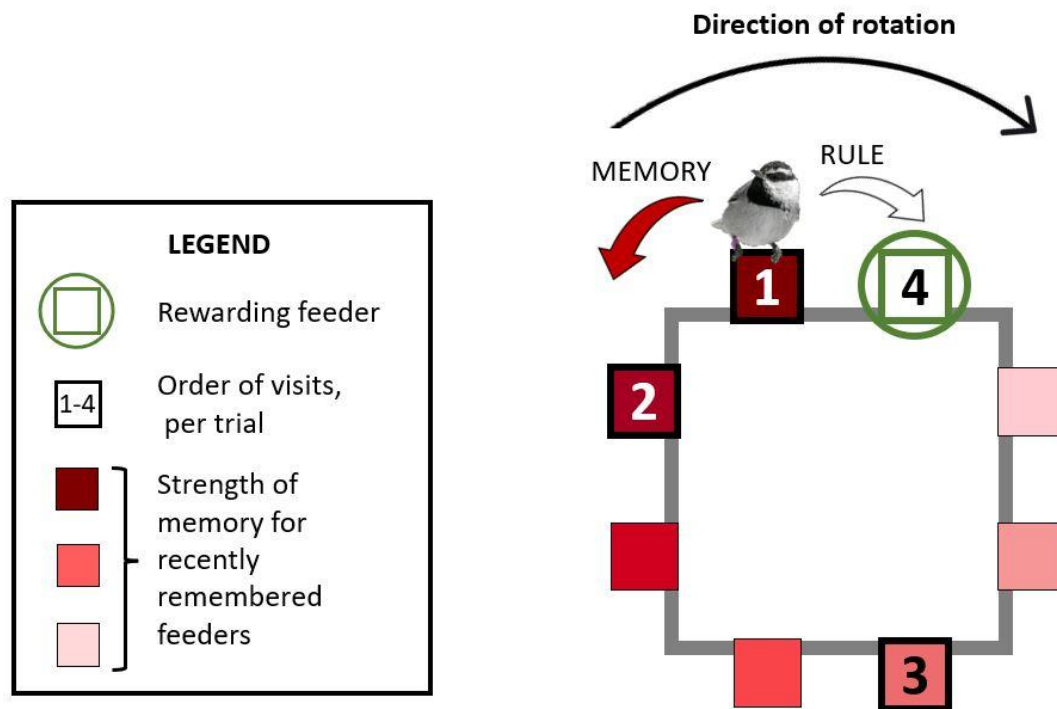


Figure 5.1. Spatial-temporal task design. Each day, the rewarding feeder (green circled square) for each bird is reassigned to the nearest feeder in the direction of rotation (e.g., clockwise). The strength of memory for recently remembered feeders (color of squares) decreases (from dark red to light red) in the opposite direction (e.g., counterclockwise). A bird starts a trial by visiting any feeder at the array and ends the trial by receiving a food reward at the correct rewarding feeder. Visits (numbered boxes) to unrewarding locations before finding the food reward are recorded as location errors. In this example, the bird started a trial by making a location error to the previous rewarding feeder from yesterday, defined as a reversal error (visit #1). After making a reversal error, the bird could search

for the rewarding feeder by relying on spatial learning and memory to move in the direction of memory recency or by using the abstract rule to move in the direction of the feeder rotation, toward the correct rewarding feeder. Moving across the array would not provide evidence for either memory recency or rule-learning. In this example, the bird moved away from the correct rewarding feeder and towards the second most recently remembered feeder (visit #2). The trial ended when it found the correct rewarding feeder on its 4th visit, after making a total of 3 location errors. See also Table S5.1.

Figure 5.2

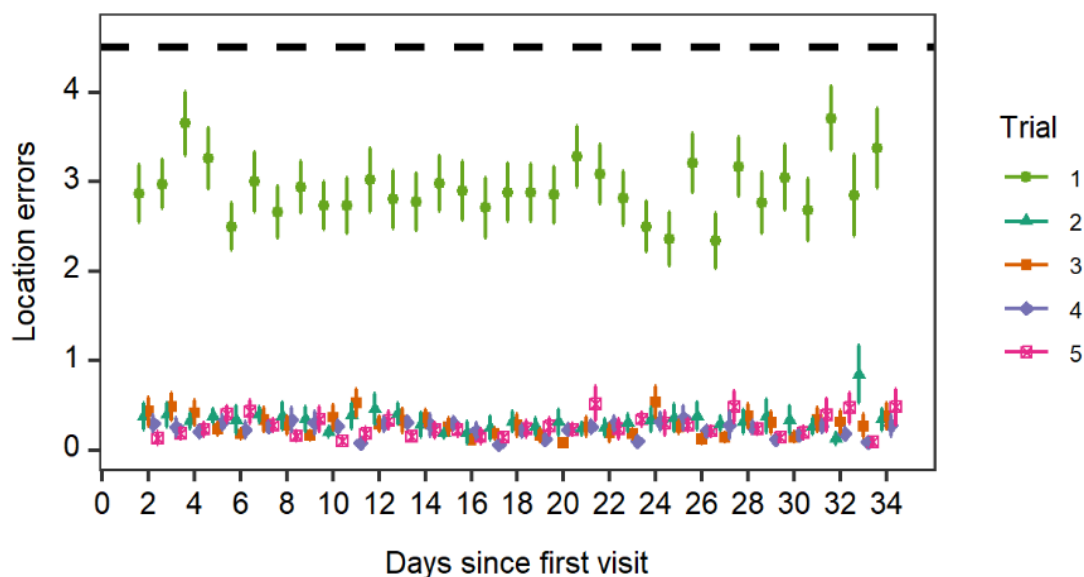


Figure 5.2. Performance in the spatial-temporal task improved across trials 1-5 but not across days. Mean location errors (points) and standard errors (error bars) per trial (color and shape). Heavy dashed line indicates performance based on chance (3.5 out of 7 location errors). See also Table S5.1 and Table S5.3.

Figure 5.3

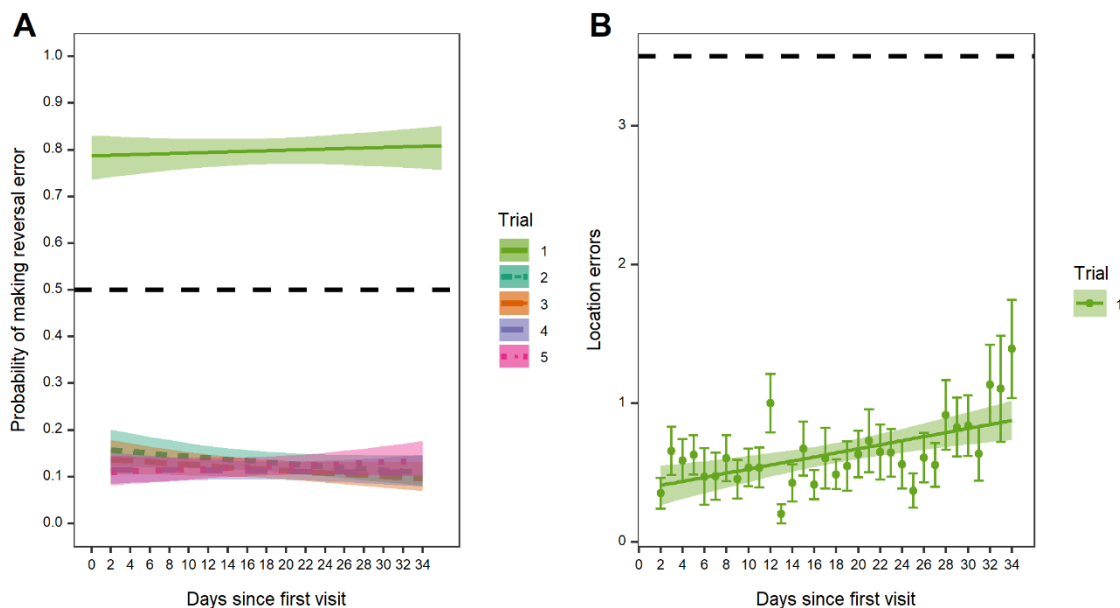


Figure 5.3. Birds did not learn to predict the temporal component of the spatial-temporal task. (a) Probability of making a reversal error (i.e., visit to previously rewarding feeder location) in trials 1-5 across days. (b) Number of location errors before making a reversal error in trial 1 across days. Regression lines with 95% confidence intervals (shaded) generated from reported GLMMs. For (b), mean location errors (points) and standard errors (error bars) per trial generated from GLMMs with an interaction of trial by day. Heavy dashed line indicates chance performance for (b), 3.5 out of 7 location errors. See also Table S5.1.

Figure 5.4

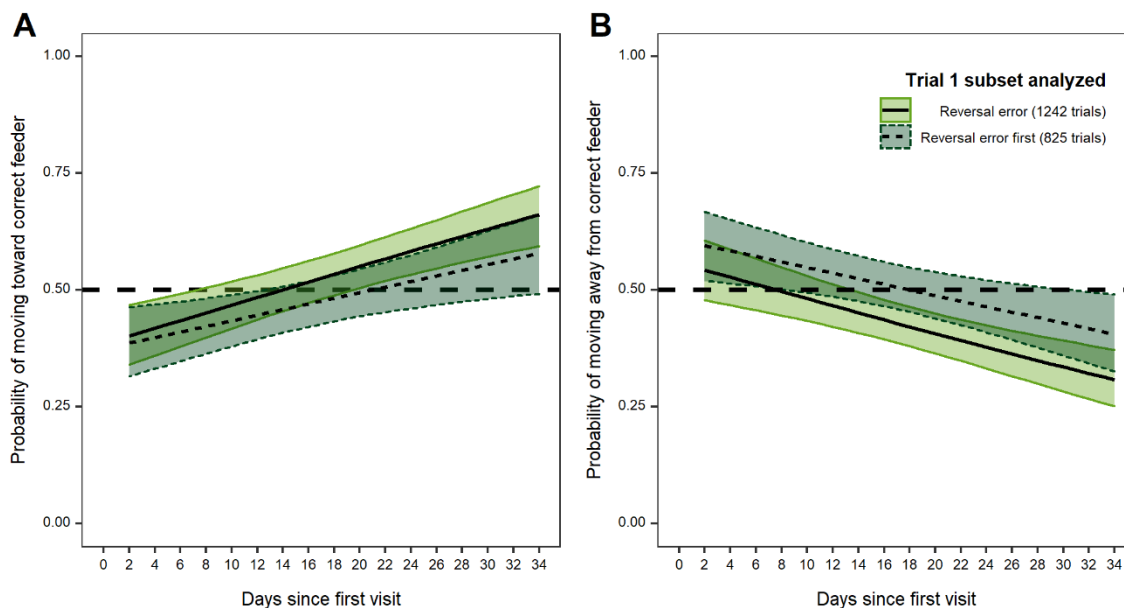


Figure 5.4. Birds were more likely to search in the direction of rotation and away from recently rewarding feeders across days. After making a reversal error in trial 1, the probability of moving (a) toward the correct feeder or (b) away from the correct feeder towards the feeders that were rewarding in the previous few days. Analysis using two subsets of trial 1: all trials with a reversal error (light green, solid line, 1242 trials) or only trials with a reversal error as the first visit (dark green, dashed line, 825 trials). Linear regression lines and 95% confidence intervals (shaded) calculated from GLMMs (ggeffects). Heavy dashed line indicates chance levels (50%). See also Table S5.1.

Star Methods***Resource Availability*****Lead contact.**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Lauren Benedict (lbenedict@nevada.unr.edu).

Materials availability.

This study did not generate new unique reagents.

Data and code availability.

- All spatial-temporal task data have been deposited at Mendeley Data Repository and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- All original code has been deposited at Mendeley Data Repository and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental Model and Study Participant Details

Study subjects and study system.

The study was conducted during the winter of 2021-2022 as part of an ongoing, long-term study of Mountain chickadees (*Poecile gambeli*) in the Sierra Nevada mountains ca. 10 km north of Truckee, CA, USA (started in 2014) at Sagehen Experimental Forest, Sagehen Creek Field Station, University of California Berkeley (Croston et al., 2017a; Rebecca Croston et al., 2016; Kozlovsky et al., 2018b; Sonnenberg et al., 2019a; Tello-Ramos et al., 2018). Wild, free-living birds were trapped and banded during annual banding efforts and breeding surveys using mist nets at feeders or by hand in nestboxes. Trapped birds were banded with unique color bands, including a passive integrated transponder (PIT)-tag with a unique alphanumeric ID (IB Technology, Leicestershire, UK). Banding efforts were concentrated at two elevations (“high”: ca. 2400m; “low”: ca. 1900m). Previous studies in this system found significant elevation-related differences in winter conditions as well as in behavioral, cognitive and morphological traits of chickadees residing at each elevation (Benedict et al., 2023a; Heinen et al., 2021; Kozlovsky et al., 2018b; Pitera et al., 2018; Sonnenberg et al., 2019a). Although we collected data at two elevations within this study system, we did not explore elevational differences in the present study because our final sample contained only one low elevation bird compared to 54 high elevation birds. We speculate this was due to low participation of low elevation birds during this particular season.

The study was approved by the University of Nevada Reno Institutional Animal Care and Use Committee (Protocol numbers 00818, 00046 and 00603) and was in

accordance with California Department of Fish and Wildlife Permit D-0011776516-4. For banding efforts, we followed the U.S. Federal Bird Banding Permit 22878. To the best of our knowledge, no birds were harmed by the collection of these data and wild-caught birds were only handled in the field for a few minutes during banding. We detected no negative effects of using PIT tags and color bands during our study. To reduce stress during handling, banding efforts were not conducted during adverse conditions (i.e., wind, precipitation).

Method Details

“Smart” feeders and spatial arrays.

Data were collected automatically using four spatial arrays of “smart” feeders (two at high elevation ca. 1.2 km apart, and two at low elevation), all previously established in 2014 (Croston et al., 2017a; Rebecca Croston et al., 2016; Heinen et al., 2021). Each array consisted of eight feeders attached equidistantly to a square aluminum frame (1.2x1.2m) that was suspended ca. 3m in the air from four trees in a small clearing (ca. 3m away from foliage on all sides). Each feeder had a perch-mounted antenna connected to a Radio-Frequency Identification (RFID) data logger to detect and record the identity and timestamp of any PIT-tagged bird that landed on the perch to forage (Bridge et al., 2019; Heinen et al., 2022). Birds could only access black oil sunflower seeds from the perch. A mechanized door controlled individual access to food within each feeder according to three different program modes: (1) “open” mode: feeder doors were always

open so all birds could forage from all eight feeders; (2) “all” mode: feeder doors were closed until any PIT-tagged bird landed on the perch and triggered the door to open, so all PIT-tagged birds could forage from all eight feeders; and (3) “target” mode: feeder doors were closed until specific PIT-tagged birds landed on the perch, so only specific PIT-tagged birds could forage from each feeder. In target mode, feeders could be programmed individually for each bird and reassigned depending on the experiment, so that at any given time birds could only access food from one of eight feeders. During all three modes, PIT-tagged birds could forage at the arrays *ad libitum* during daylight hours (ca. 06:00-20:00) until feeders automatically closed and turned off each night. When feeders were off, visits were not recorded, and birds were not able to access their rewarding feeder. However, birds were expected to stop foraging several hours before the feeders turned off (Pitera et al., 2018).

Trials during cognitive tasks.

During each cognitive task, “smart” feeders automatically collected visit data from all PIT-tagged birds (bird ID, date, time), regardless of whether the bird received a food reward. Visits could be grouped into “trials”; a trial began when a bird visited any feeder at an array and ended when the bird received a food reward at its assigned rewarding feeder (Fig. 5.1, Table S5.1). Chickadees forage for seeds one-at-a-time, leaving the array to consume or cache the seed; thus, each trial resulted in a single food reward. Chickadee motivation to complete trials during each task was not constrained by appetite (Rebecca Croston et al., 2016), as caching drive is unrelated to daily energy requirements (Bridge

et al., 2019; Pravosudov, 1985) and likely increases participation in food-rewarded tasks during caching seasons. During each trial, any incorrect feeder location that a bird visited before it found a food reward was counted as a “location error” (Table S5.1). Chickadees search the array by landing and inspecting feeders one at a time by moving from feeder to feeder until they discover the rewarding feeder (Benedict et al., 2021), so birds could make a maximum of 7 location errors per trial, as there were eight total feeder locations in an array and only one provided a food reward per bird. Birds typically arrived at the arrays in small flocks but foraged individually, using both social and asocial information to search for food (Heinen et al., 2022, 2021).

Spatial learning and memory task.

To assess individual cognitive ability, we conducted a spatial learning and memory task following our previous work (Croston et al., 2017a; Tello-Ramos et al., 2018). First, all feeders were set to “open” mode (January 10-26, 2022) and then “all” mode (January 26-February 2, 2022) to habituate the birds to the feeders at the arrays. Feeders switched to “target” mode to start the spatial learning and memory task (February 2-6, 2022). For four days, access to the feeders was restricted so that each bird could only forage from one feeder in a given array. Birds were assigned to their rewarding feeder pseudo-randomly: tagged birds were equally distributed across all eight feeders and no individual was assigned to the feeder that they had visited most frequently during “all” mode. Birds rarely visited both arrays, which were spaced ca. 1.2km apart, and so were only assigned a “target” feeder at one array. Performance was estimated as the mean number of location

errors in the first 20 trials of the task, with better performance indicated by lower values. We have previously shown that this metric is a meaningful representation of individual variation and is associated with differences in fitness (Croston et al., 2017a; Heinen et al., 2021; Kozlovsky et al., 2018b; Pravosudov, 2006; Sonnenberg et al., 2019b).

Spatial reversal learning task.

Immediately after the spatial learning and memory task, the spatial reversal learning task began by changing the target feeder for each bird to another feeder on a different side of the spatial array (February 6-10, 2022). This was a single reversal task, not a serial reversal task, so there was only one change in reward contingencies. To minimize social learning, birds were reassigned individually (Tello-Ramos et al., 2018). Performance was estimated as the mean number of location errors in the first 20 trials of the task, with better performance indicated by lower values, following our previous work (Benedict et al., 2023a; Croston et al., 2017a; Pravosudov, 2006; Sonnenberg et al., 2019b; Tello-Ramos et al., 2018).

Spatial-temporal task.

The spatial-temporal task began immediately after the spatial single reversal task (February 10 - March 16, 2022). Birds were reassigned to a new rewarding feeder overnight each day so that the food reward rotated either clockwise or counterclockwise to the next nearest feeder (Fig. 5.1). Importantly, the feeders themselves did not move;

instead, the Arduino board within each smart feeder automatically updated the list of specific birds that could access food at that feeder each day (Heinen et al., 2022). Birds were randomly assigned to either a clockwise or counterclockwise rotation, and the direction of rotation remained consistent for each bird throughout the task. Feeder assignments continued to rotate automatically for 34 days, changing overnight while feeders were closed so that each morning (starting at 06:00) birds could access the new day's rewarding feeder. Performance was estimated as the number of location errors per trial (range: 0-7) as well as whether or not the bird made a reversal error, or a location error made to the previous day's rewarding feeder.

Quantification and Statistical Analysis

Data exclusions for spatial-temporal data.

In the spatial-temporal task, 165 birds completed at least one trial during the spatial-temporal task (N = 133 from high elevation, N = 32 from low elevation). Participation during the 34-day task varied across birds, both in terms of the number of days (1-34 days) and the number of completed trials per day (0-500 trials per day). We needed to account for this variation in participation because birds could only learn the rule by first learning the sequence of daily rewarding feeders. Each bird's experience should depend on which days it visited the array during the task and whether or not it learned the daily location of the food reward. For example, a bird that participated in 5 consecutive days of the task could learn the feeder sequence "1, 2, 3, 4, 5". But a bird that participated in 5 nonconsecutive days, missing three days, would experience a different sequence of

feeders: “1, 2, 6, 7, 8”. Birds with different perceptions of the daily feeder rotation may not be able to learn the abstract rule that we expected. Birds could fail to learn the daily feeder location due to poor cognitive ability or due to low participation; as such, it was important to exclude data due to low participation before analyses.

To compare birds based on their experience with the task, we created the variable “days since first visit” to count the number of days since each bird completed its first trial during the spatial-temporal task. We defined a “missed” day as any day during the spatial-temporal task that a bird was not detected at the array at all or that the bird completed fewer than 20 trials (Table S5.1). We chose the 20-trial threshold based on our previous work using mean performance scores over the first 20 trials of the two previous spatial cognitive tasks, which have been associated with variation in fitness outcomes (Pravosudov, 2006; Sonnenberg et al., 2019b). Using 20 trials per day provided a conservative estimate to ensure that every bird had sufficient experience with the daily rewarding feeder both to learn and reinforce the spatial memory of the daily food reward. For analyses, data were excluded from days with fewer than 20 trials, as well as each day immediately following a missed day. The “days since first visit” variable increased by 1 for each day, regardless of whether data were excluded (see Fig. S5.1 for detailed examples).

Next, we made a distinction between missing two or more days in a row and only missing one day at a time. When returning to the feeder arrays after missing one day, a bird could potentially still learn the direction-rule because the next rewarding feeder would be located nearby in the correct direction. But after missing two or more

consecutive days, the next rewarding feeder could be located on the opposite side of the array, or further away, and this could provide confusing information to the bird about the spatial-temporal task. As such, we excluded data from all trials after a bird missed two or more days in a row (Table S5.2, Fig. S5.1). We only used data from *before* a bird missed two or more days in a row to make sure that we only analyzed birds when they attempted the task for the first time; partial experience with the task could affect learning performance.

Finally, we excluded data based on the total number of missed days, so that each bird never exceeded a maximum of four missed days or 15% of the total number of days analyzed in the study. When possible, we excluded data after a bird hit these maximum criteria (i.e., after the 4th missed day) instead of excluding the bird entirely (determined from the “days since first visit” counter; Table S5.2, see Fig. S5.1 for detailed examples). We also set a minimum threshold: we excluded birds that did not complete at least 16 days during the spatial-temporal task (including up to 2 missed days). To be conservative, if a bird missed a day within the last 3 days of its last day detected, we excluded all trials after that missed day.

The final dataset ($N = 55$) only contained one low-elevation bird, so elevational comparisons were dropped from the analysis. Lower participation at low elevation was not completely unexpected, as we have previously recorded low participation during the winters of 2017-2018 and 2018-2019. Overall, these exclusions were important because we could not control participation in the study and so needed another way to ensure that we only analyzed birds that had a reasonable opportunity to learn the task. It is possible

that these exclusions may have introduced bias to the analysis by selecting a specific subset of birds. However, we do not think these exclusions substantially affected our interpretations because our conclusions can only be based on birds that completed testing every day. Motivation to participate in the spatial-temporal task every day is likely related to a wide variety of factors and is likely not associated with the individual cognitive abilities we focus on in this study.

Analyses.

The spatial-temporal task dataset consisted of longitudinal data collected from a known set of marked individuals measured repeatedly across time. We analyzed these data primarily through generalized linear mixed effects models (GLMMs) to assess how variation in performance varied throughout the task. All GLMMs included days since first visit as a fixed effect (range: 2-34 days, centered and scaled) and individual bird ID as a random intercept to account for pseudoreplication and temporal autocorrelation. Continuous variables were centered and scaled to compare explanatory variables with widely varying ranges and to improve residual fit and convergence.

We analyzed data from the first trial each bird completed per day (“trial 1”), the first 2-5 trials per day (“trials 2-5”) and the last 5 trials per day separately to address different hypotheses. When a bird makes a reversal error during trial 1, the bird should learn that the reward contingency has changed and the direction the bird decides to move next should indicate where the bird expects to find food. This decision happens before receiving any reinforcement and should provide support for whether or not the bird

learned the abstract rule. In contrast, behavior during all other trials after trial 1 reflects the bird's ability to overcome proactive interference and learn the new daily feeder location. We performed a specific analysis for the first 2-5 and not subsequent trials because we have previously found that birds learn new rewarding feeder locations extremely quickly (Benedict et al., 2023a; Tello-Ramos et al., 2018) and we were interested in variation in the initial stages of the learning process. We analyzed data from the last five trials completed by each bird each day to confirm that the bird successfully learned the correct rewarding feeder, and so could be reasonably expected to learn the abstract rule. There was no overlap between the first and last 5 trials analyzed, since all birds completed at least 20 trials per day.

We calculated several metrics to explore our hypotheses: location errors, reversal errors, location errors before a reversal error and direction moved after making a reversal error. Location errors measure an individual's overall ability to learn the rewarding feeder location during the spatial-temporal task, whereas reversal errors measure the ability to learn new reward contingencies while inhibiting a response to the previously learned feeder location (Izquierdo et al., 2017; Tello-Ramos et al., 2019). While some studies analyze repeated visits to a previously rewarding location to estimate reversal errors, we defined reversal errors as whether or not the bird visited the previous reversal location during a given trial. Birds could make a maximum of one reversal error per trial because there was only one feeder location providing a food reward on the previous day. This allowed us to use logistic regressions to analyze reversal errors across time during the spatial-temporal task. We were also interested in behavior before and after birds made a reversal error. The number of location errors before making a reversal error provided an

estimate of whether or not birds went directly to the previous day's rewarding feeder at the beginning of a trial. This metric allowed us to assess to what extent birds were learning to predict or to retain memories of the previous feeder location. The direction a bird moves after making a reversal error provides information about where the bird expects to find food after conditions have changed and what strategy the bird may be using.

Model fixed effects.

To control for variation in reinforcement of the previous day's rewarding feeder, GLMMs that used metrics from the first 5 trials per day also included a fixed effect for the total number of trials completed the previous day ("previous day trials", range: 20-500, centered and scaled; Table S5.1). In models that included previous day trials as a fixed effect, we excluded the first day of the task, as there were no previous day trials reported. To explore the effects of spatial learning and memory ability and spatial reversal learning ability on performance in the spatial-temporal task, we fit separate GLMMs using the subset of birds that participated in all three cognitive tasks (Table S5.2). We used the mean location errors per trial in the first 20 trials of the spatial learning and memory task (range: 0.05-1.35 mean location errors per trial, scaled and centered) and for the spatial reversal learning task (range: 0.15-1.05 mean location errors per trial, scaled and centered).

Overall performance in trial 1 and trials 2-5

To explore how performance on the spatial-temporal task varied across days, we fit a GLMM (Poisson distribution) to the number of location errors in trial 1 each day (range: 0-7 location errors) with fixed effects of days since first visit and previous day trials. To explore the effects of the other two cognitive tasks on trial 1 performance, we fit two additional GLMMs by adding either a fixed effect of spatial learning and memory performance or spatial reversal learning performance.

We compared mean performance in trial 1 (location errors) to chance level using one-sample student t-tests. These tests were aimed to determine whether birds were searching randomly during the first trial each day or whether they appeared to use learning and memory ability to find the correct feeder. Given eight choices, random expectation for location errors in one trial is equal to 4.5 errors (Branch et al., 2022). Regarding the assumptions of the student t-test, these data were (a) continuous, (b) normally distributed, (c) represented a moderate sample size of $N = 55$ and passed a simple test for homogeneity of variance. To ensure that data were independent, we run separate t-tests per day to avoid repeat measures of individuals (test statistics in Table S5.3). Multiple comparisons may inflate the risk of Type I errors, so caution should be used to analyze these results. The Bonferroni-adjusted threshold for significance is $\alpha = 0.0015$, after dividing the original alpha value (0.05) by the number of tests ($N = 34$).

Models for learning to predict the temporal component.

To assess whether birds learned to predict the location of the correct rewarding feeder each day, we explored whether the probability of making a reversal error in trial 1 varied across days. If birds learned to predict during the spatial-temporal task, we expected to see an overall decrease in both the number of location errors and the probability of making a reversal error, suggesting that birds went directly to the correct feeder during trial 1 each day. We created a binary categorical response variable for whether or not a bird made a reversal error in trial 1 and fit a GLMM (binomial distribution, “logit link”, reported in tables using log-odds scale) with the fixed effects of days since first visit and previous day trials. To explore the effects of the other two cognitive tasks on the probability of making a reversal error in trial 1, we fit two additional GLMMs by adding either a fixed effect of spatial learning and memory performance or spatial reversal learning performance.

To assess whether birds continued to make reversal errors during trials 2-5, we fit another GLMM (binomial distribution, “logit link”) with the probability of making a reversal error per trial as the response variable. We included fixed effects of days since first visit, trial number (categorical, 4 levels), previous day trials and an interaction effect between days and trial number. We included trial number as a fixed effect because we were interested in the differences in effect of each trial, as performance should continue to improve across trials.

We fit another model to estimate the number of location errors made before a reversal error in trial 1 each day. This should provide information about whether birds

were making a reversal error first during the task, which would suggest they remembered where the previous day's food reward had been located. This only used the subset of trials in which birds made a reversal error, so this does not represent performance or likelihood of making a reversal error. We fit a GLMM (negative binomial distribution) using the MASS package (Venables and Ripley, 2002) to these location errors with fixed effects of days since first visit and previous day trials.

Models for learning the direction rule.

To assess whether the birds learned the abstract rule of feeder rotation, we scored each trial for the direction the bird moved after making a reversal error in trial 1. A trial was scored "toward" if the bird moved toward the correct feeder location, in the same direction as the daily feeder rotation and was scored "away" if the bird moved away from the correct feeder, toward the most recently rewarding feeders during the task. For example, consider that the feeders are numbered 1-8, increasing in the direction of rotation, and a bird was assigned to rotate clockwise around the arrays, starting with feeder 1 on day 1. In the first trial completed on day 2, this bird makes a reversal error by visiting feeder 1, the previous day's rewarding feeder (Fig. S5.1). The direction for this trial would be scored depending on the next feeder that the bird visited: "away" for moving backwards to feeders 8 and 7; "toward" for moving forward in the correct direction of rotation to feeders 2, 3, or 4; and "unscored" for feeders across the array (feeders 6 and 5). We scored direction for two subsets of the data: a more conservative subset, in which a bird made a reversal error in trial 1 for their first visit of the trial, and a

less conservative subset, in which a bird made a reversal error in trial 1 at any point during the trial. We tested the less conservative subset because if birds learned the direction-rule, then we would expect them to learn to move in the correct direction regardless of whether they remembered to go to the previous day's rewarding feeder first.

We used the direction data to make two binary category variables, one to indicate whether or not the bird went "toward" and one to indicate whether the bird went "away" from the correct rewarding feeder. We then fit GLMMs (binomial, "logit link") with fixed effects of days since first visit and the number of trials completed the previous day, one for the "toward" and one for the "away" response variables. We fit two models using each data subset.

Model for the last 5 trials per day.

To assess whether individuals learned each day's rewarding feeder location and assess how this performance varied across days, we calculated the sum of location errors in the last 5 trials completed each day. We fit a GLMM (Poisson distribution) to the sum of location errors in the last 5 trials with a fixed effect of days since first visit (see also Fig. S5.2).

Statistical software.

All analyses were performed using R version 4.1.0 (R Core Team, 2021). (G)LMMs were fit using *lme4* (Bates et al., 2015) unless otherwise indicated and statistics were reported

using Wald Chi-Squared values from analysis of variance (ANOVA) tests fit with *car* (Fox and Weisberg, 2019). GLMMs with a negative binomial distribution were fit with the *MASS* package (Venables and Ripley, 2002). Regression assumptions and goodness-of-fit were evaluated using *DHARMA* (Hartig, 2021). Residual variance was reported separately for fixed effects and the full model as marginal ($R^2_{GLMM(m)}$) and conditional ($R^2_{GLMM(c)}$) residual variance for GLMMs, respectively, following (Nakagawa and Schielzeth, 2013) and calculated in the *performance* package (Ludecke et al., 2020). Estimated marginal means and associated standard errors were calculated with *emmean* (Lenth, 2020) and *ggeffects* (Ludecke, 2018) to make within-group comparisons, plot model estimates and report Tukey post hoc analyses. All plots were made with *ggplot2* (Wickham, 2016).

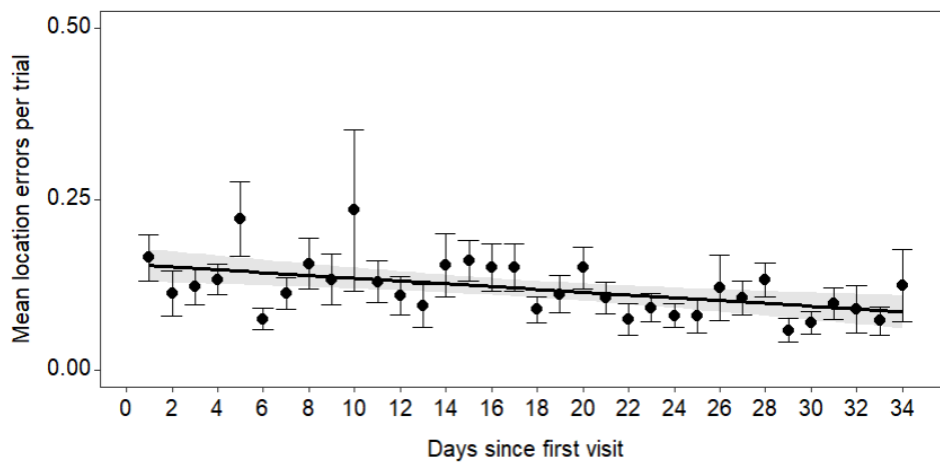
Supplement

Supplemental Figure Legends

Figure S5.1. Examples of excluded data (grey) and included (green) data from spatial-temporal task, Related to STAR Methods. Columns indicate the number of days since a bird's first visit during the task (relative for each bird, white numbers). Rows (a-f) represent example data from 6 individuals. Missed days (white numbered boxes) were days when a bird completed fewer than 20 trials or did not visit the feeder array at all and thus may not have learned the daily feeder location. Thick red markings indicate the reason data were excluded. Birds were excluded entirely for missing two consecutive days (red "XX") within the first 16 days (a, b) or for missing more than four total nonconsecutive days or more than 15% of the total days since first visit ("c"). Data were truncated after two missed days (d), after exceeding the number of permitted missed days (e), or if one of the last three days was missed (f).

Figure S5.2. Mean location errors per trial over the last 5 trials decreased across days, Related to STAR Methods. Means (points), standard errors (error bars) and regression line with 95% confidence intervals (shaded) were initially defined using the R package *ggplot2*.

Figure S5.2



Supplemental Tables

Table S5.1. Terms and definitions, Related to STAR Methods, Figure 5.1, Figure 5.2, Figure 5.3 and Figure 5.4.

Term	Definition or usage
Rule	Generalizable concepts that have been learned from previous experiences and can be transferred to new situations
Abstract concept learning	Learning a rule based on the relationships between or among stimuli and learning to generalize this rule to novel stimuli ^{S1} . Abstract concepts (also “relational concepts”) group stimuli based on relations, not based on simple similarity (i.e., not based on physical similarity or similar outcomes) ^{S2}
Executive functions	A group of top-down cognitive processes, considered part of higher-level cognition, involved with integrating different types of information for goal-oriented behaviors. Following Diamond (2013), there are three general categories : inhibition, working memory and cognitive flexibility ^{S3} . Also called “executive control functions” in animal behavior studies in which specific neural processes are not studied ^{S4} .
Daily feeder rotation	The pattern used in the spatial-temporal task, in which each bird’s assigned rewarding feeder was reassigned every day. The new rewarding feeder would appear to rotate around the square feeder array, always to the nearest feeder to the left or right, in the same direction for the entire task.
Rewarding feeder	The location of the food reward within the 8-feeder array. Assigned individually for each bird. I.e., “target feeder”, “assigned feeder”, “correct feeder” or “feeder assignment”
Trial	Begins when a bird visits any feeder at an array and ends when a bird obtains a food reward
Location error	Number of unrewarding feeder locations visited in a trial (max: 7)
Reversal error	In this study, a location error to the most recently rewarding feeder, from the previous day (maximum per trial: 1)
Trial 1	The first trial completed per bird per day. This is always the first trial after the daily feeder rotation, so typically in the morning.
Days since first visit	A relative metric for each bird, counting days since the first completed trial of the spatial-temporal task. Does not correlate with absolute date
Missed day	When a bird was not detected at all or completed fewer than 20 trials on a given day during the spatial-temporal task
Previous day trials	The number of trials completed the previous day. Included in models to control for total learning and experience with the feeder location that previously provided a food reward

Table S5.2. Sample sizes after each exclusion, related to STAR Methods.

Datasets after each exclusion	Sample size		
	High	Low	Total
All birds with at least 1 trial	133	32	165
Completed 16 days with 20 trials per day before missing 2 consecutive days	62	1	63
With fewer than 15% skips	60	1	61
With no more than 1 day of partial experience before task*	54	1	55
With learning and memory performance*	48	1	49
With reversal learning performance*	46	1	47

*Main datasets used for analyses

Table S5.3. Statistics from one-sample student t-tests for performance in trial 1 of spatial-temporal task, Related to Figure 2. Mean trial 1 performance per day (mean location errors), t-statistics (t), unadjusted P-values (P) and 95% confidence intervals (CI) displayed. The Bonferroni-adjusted threshold for significance is $\alpha = 0.0015$, after dividing the original alpha value (0.05) by the number of tests ($N = 34$). Day refers to days since first visit per bird, not actual date of experiment.

Day	Mean	t	df	95% CI	P
2	2.86	-5.07	36	[2.21, 3.52]	<0.001
3	2.98	-5.49	42	[2.42, 3.54]	<0.001
4	3.65	-2.39	42	[2.93, 4.37]	0.02
5	3.26	-3.65	41	[2.58, 3.95]	<0.001
6	2.50	-7.54	43	[1.97, 3.03]	<0.001
7	3.00	-4.45	49	[2.32, 3.68]	<0.001
8	2.66	-6.39	49	[2.08, 3.24]	<0.001
9	2.94	-5.42	48	[2.36, 3.52]	<0.001
10	2.73	-6.54	48	[2.19, 3.28]	<0.001
11	2.73	-5.68	48	[2.11, 3.36]	<0.001
12	3.02	-4.12	47	[2.3, 3.74]	<0.001
13	2.80	-5.17	50	[2.15, 3.46]	<0.001
14	2.77	-5.33	52	[2.12, 3.42]	<0.001
15	2.98	-4.92	52	[2.36, 3.6]	<0.001
16	2.90	-4.78	50	[2.23, 3.57]	<0.001
17	2.71	-5.28	47	[2.03, 3.39]	<0.001
18	2.88	-4.98	49	[2.23, 3.53]	<0.001
19	2.88	-5.09	48	[2.24, 3.52]	<0.001
20	2.85	-5.18	47	[2.21, 3.49]	<0.001
21	3.28	-3.60	45	[2.6, 3.96]	<0.001
22	3.09	-4.32	46	[2.43, 3.74]	<0.001
23	2.82	-5.67	48	[2.22, 3.41]	<0.001
24	2.50	-6.92	49	[1.92, 3.08]	<0.001

Day	Mean	t	df	95% CI	P
25	2.36	-7.02	46	[1.75, 2.98]	<0.001
26	3.21	-3.86	46	[2.54, 3.88]	<0.001
27	2.34	-6.92	46	[1.71, 2.97]	<0.001
28	3.17	-3.99	46	[2.5, 3.84]	<0.001
29	2.76	-5.08	41	[2.07, 3.45]	<0.001
30	3.05	-3.93	40	[2.3, 3.8]	<0.001
31	2.68	-5.24	40	[1.98, 3.38]	<0.001
32	3.71	-2.22	37	[2.99, 4.43]	0.03
33	2.85	-3.62	32	[1.92, 3.78]	0.001
34	3.38	-2.52	28	[2.47, 4.29]	0.02

CI, confidence interval; DF, degrees of freedom. Bolded values indicate significance ($\alpha < 0.05$).

Supplemental References

Bobrowicz, K., and Greiff, S. (2022). Executive functions in birds. *Birds*, 3:184–220.
10.3390/birds3020013.\

Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, 64:135–168.
10.1146/annurev-psych-113011-143750.

Katz, J.S., Wright, A.A., and Bodily, K.D. (2007). Issues in the comparative cognition of abstract-concept learning. *Comparative Cognition and Behavior Reviews*, 2:79–92. 10.3819/ccbr.2008.20005.

Lazareva, O.F., and Wasserman, E.A. (2017). Categories and Concepts in Animals. In *Learning and Memory: A Comprehensive Reference* (Elsevier), pp. 111–139.
10.1016/B978-0-12-809324-5.21008-0.

Conclusions

Overall, mountain chickadees living in harsh environments are capable of a diverse and impressive range of cognitive abilities and information-updating behaviors. While searching for food within a familiar landscape, chickadees adjusted their search behavior in relation to the harshness and predictability of recent environmental conditions, increasing visits for information maintenance when birds experienced harsher and less predictable environments (Chapter 2). Having the most updated information may be beneficial in case preferred food sources were to become unavailable and birds needed to quickly switch to a new food source. Furthermore, chickadees learned and used complex spatial and spatial-temporal rules while making foraging decisions. Individuals learned both the serial reversal rule “win-stay, lose-shift” to track a food source that alternated predictably between two locations within an array (Chapter 4) and also learned an abstract, relational rule to track a food source that shifted in the same direction to a new feeder at the array each day (Chapter 5).

In conclusion, this dissertation presents some of the first evidence of abstract rule-learning in wild, nonhuman animals and suggests that chickadees are capable of using cognitive processes associated with executive control functions to forage more efficiently in the wild. Spatial learning and memory ability associated with cache retrieval has been extensively researched in chickadees, and more broadly in Paridae. But many cognition-based behaviors integrate multiple cognitive traits, and potentially involve executive control functions, which have been less well documented in nonhuman animals. As such, the results from this dissertation provide important insight into the ways spatial learning

and memory, cognitive flexibility, and rule-learning interact and contribute to foraging-based cognitive strategies in a wild, free-living bird.