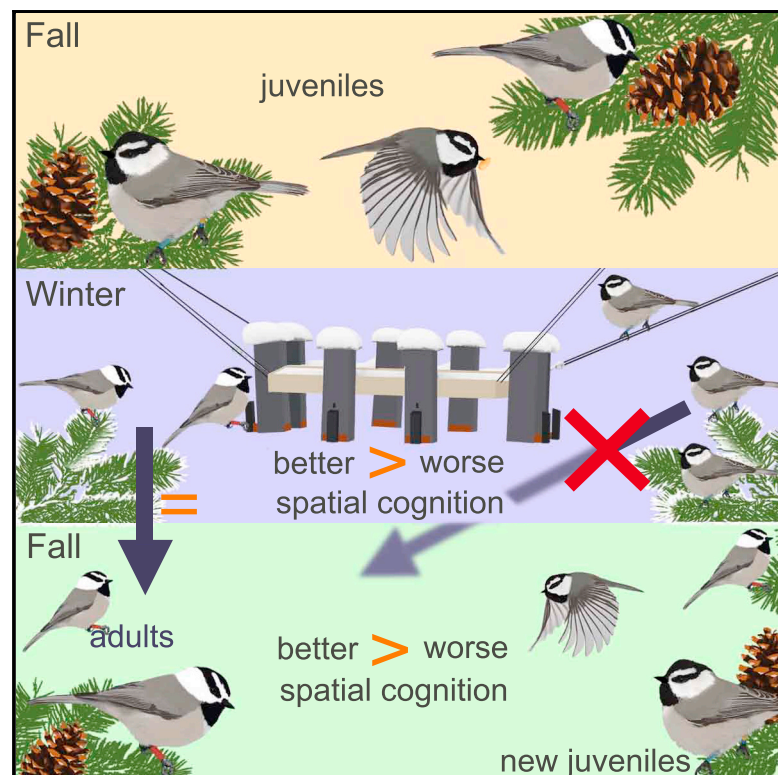


Current Biology

Natural Selection and Spatial Cognition in Wild Food-Caching Mountain Chickadees

Graphical Abstract



Highlights

- Food-caching chickadees rely on spatial cognition to recover caches
- Adult chickadees perform better than juveniles in spatial cognitive tasks
- Performance in spatial cognitive tasks does not differ between seasons
- Variation in spatial cognitive abilities is associated with differences in survival

Authors

Benjamin R. Sonnenberg,
Carrie L. Branch, Angela M. Pitera,
Eli Bridge, Vladimir V. Pravosudov

Correspondence

vpravosu@unr.edu

In Brief

Sonnenberg et al. present first evidence for natural selection acting on spatial cognitive abilities in food-caching chickadees by showing that survival was associated with better spatial cognitive performance in juvenile birds, that adults performed better than juveniles and that cognitive performance did not change between seasons.

Natural Selection and Spatial Cognition in Wild Food-Caching Mountain Chickadees

Benjamin R. Sonnenberg,¹ Carrie L. Branch,^{1,2} Angela M. Pitera,¹ Eli Bridge,³ and Vladimir V. Pravosudov^{1,4,*}

¹University of Nevada Reno, Department of Biology and Ecology, Evolution, and Conservation Biology Graduate Program, Reno, NV 89557, USA

²Cornell Lab of Ornithology, Cornell University, 159 Sapsucker Woods Rd, Ithaca, NY 14850, USA

³University of Oklahoma, Oklahoma Biological Survey, 111 E. Chesapeake St., Norman, OK 73019, USA

⁴Lead Contact

*Correspondence: vpravosu@unr.edu

<https://doi.org/10.1016/j.cub.2019.01.006>

SUMMARY

Understanding how differences in cognition evolve is one of the critical goals in cognitive ecology [1–5]. In food-caching species that rely on memory to recover caches, enhanced spatial cognition has been hypothesized to evolve via natural selection [2, 6–8], but there has been no direct evidence of natural selection acting on spatial memory. Food-caching mountain chickadees living at harsher, higher elevations, with greater reliance on cached food have better spatial learning abilities and larger hippocampi containing more and larger neurons compared to birds from milder, lower elevations [9, 10]. Here, we tested for natural selection on spatial cognition in wild food-caching mountain chickadees at high elevations and documented the following: (1) compared to first-year juveniles, adults showed significantly better performance on two spatial cognitive tasks—spatial learning and memory and a consecutive reversal learning task; (2) cognitive performance in both spatial learning and reversal learning tasks was not significantly different between years in the same chickadees tested in their first year of life and after surviving to their second winter; and (3) cognitive performance in the spatial learning task was significantly better among the first-year juveniles that survived to their second winter compared to the subset of juveniles that did not survive. Taken together, our results provide evidence for natural selection on spatial cognition in a food-caching species living in harsh environments and suggest that natural selection associated with local environmental conditions might be generating intraspecific differences in cognitive abilities.

RESULTS

Cognitive abilities are known to vary both among and within species [1–5], and it has been hypothesized that both inter- and intra-specific differences in various cognitive traits have evolved

via natural selection, with better cognitive performance associated with higher fitness [2]. Most evidence of natural selection shaping existing variation in cognition comes indirectly from comparative studies [1–8], while direct evidence in wild populations remains elusive [4, 5].

Scatter-hoarding species present a convenient model to investigate natural selection on cognition because they store large numbers of food items during autumn, when naturally available food is plentiful, and rely on these caches for overwinter survival [2, 6–8]. The importance of food caches for overwinter survival depends on environmental conditions, with harsher winter conditions associated with greater dependence on food caches for survival [2, 11]. It has been well established that most scatter-hoarding species use spatial memory to retrieve stored food [2, 8]; therefore, individual variation in spatial learning and memory ability should result in variation in cache retrieval success and potentially survival.

We previously tested this hypothesis by comparing spatial cognition and associated brain morphology among populations of food-caching black-capped chickadees (*Poecile atricapillus*) along both longitudinal and latitudinal gradients of winter climate severity across North America [11–17] and in mountain chickadees (*P. gambeli*) along an elevational gradient of winter climate severity [9, 10, 17]. In both species, chickadees from harsher environments showed greater food-caching propensity, better spatial learning and memory, and larger hippocampus, a brain region involved in spatial cognition, with significantly more and larger neurons compared to chickadees from milder environments [2]. Differences in spatial cognition among populations appear to have a heritable basis, as they persisted in a common garden experiment [15] and were associated with differential gene expression in the hippocampus of chickadees reared and maintained in the same controlled environment [18]. Direct evidence in mammals also suggests that spatial memory and hippocampus volume are heritable [e.g., 19] and therefore available for selection.

Here, we investigated whether there is natural selection on spatial cognition in wild mountain chickadees at high, harsh elevations in the Sierra Nevada mountains, USA. Despite elevation-related differences in spatial cognition [9, 10, 17], we found no evidence for genetic population structure between birds from high (harsher winters) and low (milder winters) elevations, suggesting some degree of gene flow [20] and even low levels of gene flow can prevent local populations from reaching an

Spatial cognitive performance

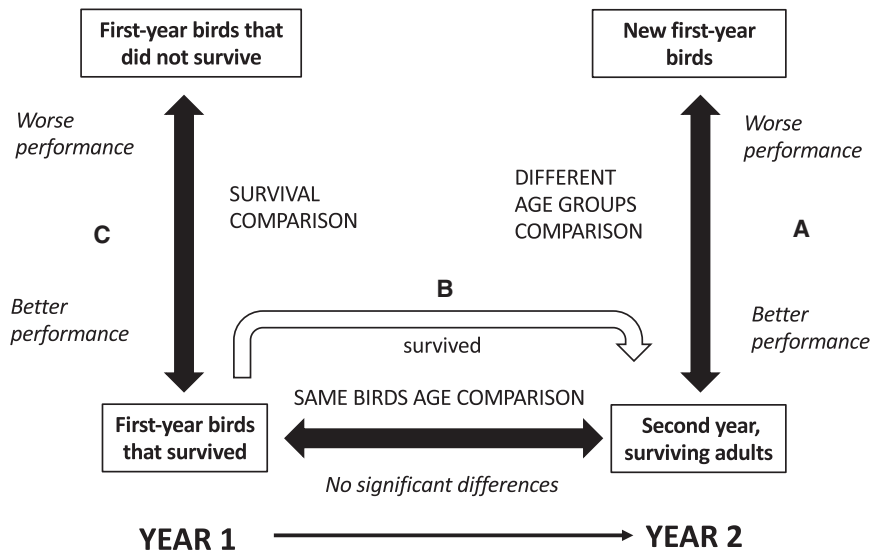


Figure 1. Diagram of the Comparisons Used in This Study

(A–C) (A) comparing adults and first-year juveniles; (B) comparing the same individuals which were first tested as first-year juveniles and then next year as surviving adults; (C) comparing individuals that survived until next year and the ones that died within the same cohort of first-year juveniles.

be evident between first-year birds and adults. Thus, if there is selection on spatial cognition, we can expect differences in cognitive performance between first-year and adult chickadees (Figure 1A).

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

were tested as first-year juveniles and the following year as adults (Figure 1B).

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

These comparisons were conceived to reveal the presence of selection on two spatial cognition metrics—spatial learning and memory ability and reversal spatial learning and memory ability (when each bird was re-assigned to a new and different rewarding feeder immediately following the spatial learning task) [22, 23]. Spatial learning and memory ability are expected to reflect the ability to remember and recover previously made food caches [2, 8], while reversal learning ability is expected to reflect learning and memory flexibility, which may be important for foraging success in unpredictable environments [23, 25]. We used mean number of location errors per trial over the first 20 trials (initial learning and memory phase) and the mean number of location errors per trial over the entire 4-day task (overall task performance measuring learning and longer-term memory) [21–23]. As birds learn, they are expected to make fewer and fewer location errors with each trial; thus, the mean number of location errors per trial over the entire task is a reliable and accurate estimate of learning and memory that is repeatable across years within individuals [21–23]. Faster learning and better memory are indicated by fewer location errors per trial, so lower mean number of location errors per trial over the entire learning task indicates better learning and memory. We used the total number of trials completed during each cognitive task as a covariate in all analyses of cognitive performance to control for individual variation in timing and frequency of trials, which allows testing whether groups differed in cognitive performance independently of potential differences in number of trials [21–23].

equilibrium. Although various evolutionary mechanisms could be at play in the population, we expect directional positive selection to be pervasive because fitness benefits for spatial cognition in a caching species are expected to be especially strong at harsh, high elevations, where reliance on food caches for overwinter survival is greater. However, variation in spatial cognitive abilities at high elevations may be maintained when winters are milder and selection is relaxed.

We developed and implemented a radio frequency identification (RFID)-based system to test spatial cognition in wild, passive integrated transponder (PIT)-tagged chickadees [21–23]. RFID-enabled feeders can be programmed to provide food only to specific individuals while recording the PIT-tag ID and time of each visit by all tagged birds. By assigning each bird to a single rewarding feeder (different feeders for different birds) within an 8-feeder spatial array, we can test spatial learning and memory performance by measuring the number of nonrewarding feeders visited (location errors) prior to landing on the rewarding feeder over multiple trials (a trial starts when a bird visits any feeder in the array and ends with a visit to the rewarding feeder). When a bird visits a rewarding feeder, a door opens allowing access to food, while any other feeder would record the ID and time of visit without providing food. We followed the same testing protocol for the last two years [23], which allowed direct comparisons of (a) cognitive performance between individuals in the same cohort of first-year birds who survived their first winter and those that did not and (b) cognitive performance between age classes—adults versus first-year birds.

We employed three well-established methods to detect natural selection. First, if selection on a particular trait occurs at a certain age, the trait frequency distribution can be expected to differ between age classes (age class comparison) [24]. In mountain chickadees, the largest overwinter mortality occurs during their first winter, so selection on survival-associated traits should

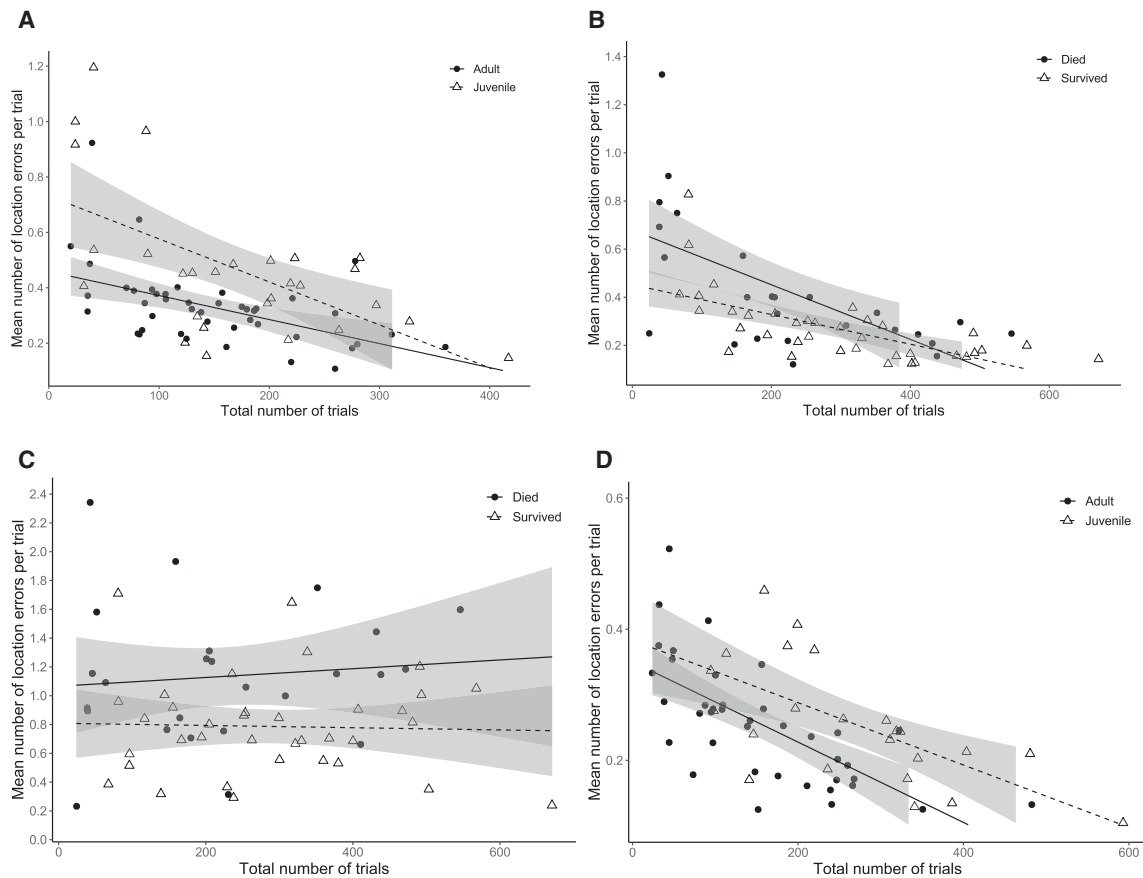


Figure 2. Performance in Spatial Learning and Memory Task and in Spatial Reversal Learning Task in Juveniles and Adults and in Juvenile Chickadees that Survived and Those that Died

(A) Spatial learning and memory task—age class comparison within the same season: mean number of location errors per trial and the total number of trials during the entire spatial learning task in the first-year birds and in adults during 2017–2018 season. See also [Figure S1](#).

(B) Spatial learning and memory task—survival comparison: mean number of location errors per trial and the total number of trials during the entire spatial learning task in first-year birds that either survived or died after the 2016–2017 testing season. See also [Figure S2](#).

(C) Spatial learning and memory task—survival comparison: mean number of location errors per trial over the first 20 trials and the total number of trials during the entire spatial learning task in first-year birds that either survived or died after the 2016–2017 testing season. See also [Figure S2](#).

(D) Reversal spatial learning and memory task—age-class comparison: mean number of location errors per trial and the total number of trials over the entire reversal spatial learning task in first-year, juvenile birds and in adults during 2017–2018 season.

Shaded areas in all graphs are 95% CI.

Spatial Learning Task

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

$F_{1,70} = 5.59$, $p = 0.02$; age effect: $F_{1,70} = 11.44$, $p = 0.001$; [Figure S1](#)).

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

Within the 2016–2017 first-year chickadee cohort (e.g., juveniles), individuals that survived and were tested again as adults ($n = 37$) in 2017–2018 performed significantly better in the spatial learning task than members of their first-year cohort that did not survive ($n = 25$) after their first year of testing (mean number of location errors per trial over the entire task: GLM, $F_{1,59} = 4.60$, $p = 0.036$; total number of trials as a covariate: $F_{1,59} = 34.4$, $p < 0.001$; effect size: Glass's $\Delta = 0.6$, [Figure 2B](#)). Cognitive performance in the spatial learning task was a significant predictor

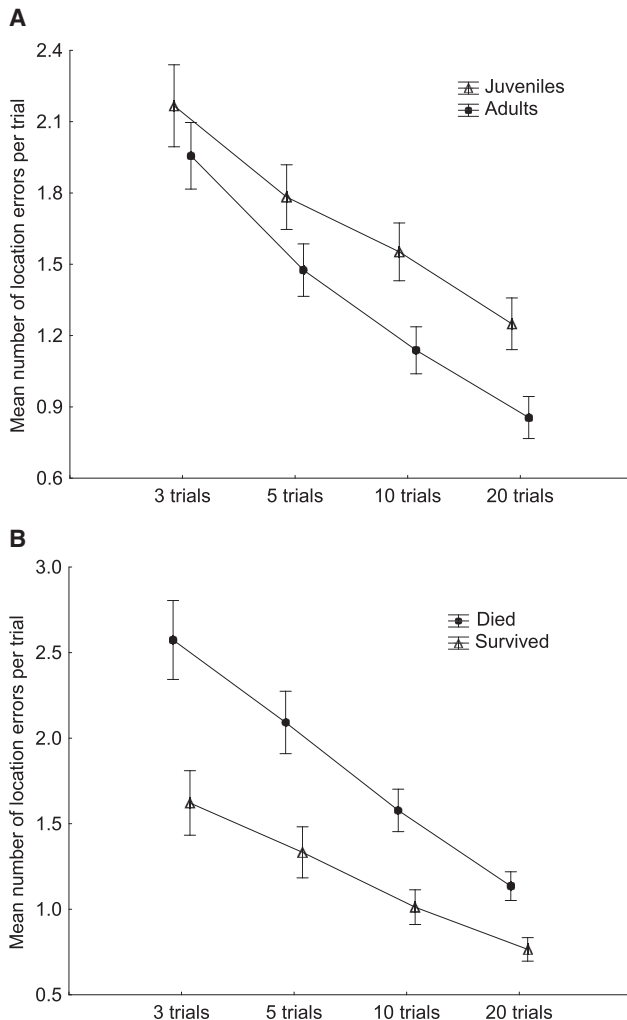


Figure 3. Spatial Learning and Memory Performance within the First 20 Trials in Adult and Juvenile Chickadees and in Chickadees that Survived and Those that Died

(A) Mean number of location errors (least-squares means and SE) per trial during the first 3, 5, 10, and 20 trials of the spatial learning task: comparing juveniles and adults within the same season (repeated-measures GLM, age: $F_{1,71} = 4.61$, $p = 0.03$; trials: $F_{3,213} = 92.2$, $p < 0.0001$).

(B) Spatial learning and memory task—mean number of location errors per trial (least-squares means and SE) during the first 3, 5, 10, and 20 trials of the spatial learning task: comparing juveniles that survived until next year with the ones that died (repeated-measures GLM, survived versus died: $F_{1,59} = 12.02$, $p < 0.001$; trials: $F_{3,177} = 22.6$, $p < 0.001$).

of survival in first-year chickadees tested in the 2016–2017 season (mean number of location errors per trial over the entire task: logistic regression: $\beta = -2.24 \pm 0.78$, $\text{Wald} = -3.77$, $p = 0.003$). Significant differences in performance between the juveniles that survived until the next year and ones that did not were detected in the first 20 trials of the spatial learning task which represents the initial learning and memory phase (GLM, mean number of location errors per trial: $F_{1,59} = 11.3$, $p = 0.001$; total number of trials as a covariate: $F_{1,59} = 0.001$, $p = 0.94$; effect size: Glass's $\Delta = 0.88$; Figure 2C). Performance during the first 20 trials of the spatial learning task was also a sig-

nificant predictor of survival (logistic regression: $\beta = -2.42$, $\text{Wald} = 8.22$, $p = 0.004$). Differences in performance between survivors and birds that later died were also present within the first 20 trials, when birds showed significant improvement between the first 3, 5, 10, and 20 trials, with the mean number of location errors decreasing with the number of trials (Figure 3B). Chickadees continued to improve their performance beyond the first 20 trials, as the mean number of location errors per trial over the entire task was significantly lower than the mean number of location errors per trial during the first 20 trials (repeated-measures GLM, $F_{1,59} = 21.3$, $p < 0.0001$, survived versus died: $F_{1,59} = 10.46$, $p = 0.001$; Figure S2).

Reversal Spatial Learning Task

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

There were, however, no significant differences in reversal spatial task performance between chickadees that were tested in 2016–2017 as first-year juveniles and survived until the next year and those juveniles that tested in 2016–2017 but did not survive (mean number of location errors per trial over the entire task; $F_{1,57} = 0.78$, $p = 0.38$; total number of trials as a covariate: $F_{1,57} = 30.9$, $p < 0.001$). All birds improved their performance throughout the task, as the mean number of location errors per trial over the entire task was significantly lower than the mean number of location errors per trial during the first 20 trials (repeated-measures GLM, $F_{1,57} = 26.3$, $p < 0.0001$).

Number of Trials

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

DISCUSSION

Our results provide evidence for natural selection on spatial cognition in wild food-caching mountain chickadees. The strongest support comes from spatial learning and memory performance, as results from all three comparisons are consistent with natural selection: (1) adults showed better spatial learning

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

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

and memory performance than first-year juveniles; (2) there were no significant differences in performance of the same cohort of chickadees that were tested as first-year birds and then as adults, i.e., they did not improve performance with experience; and (3) spatial cognitive performance was a significant predictor of survival in first-year juvenile chickadees tested in 2016–2017; birds that survived until the 2017–2018 season showed significantly better performance in the spatial learning and memory task compared to birds that died.

We also found large age-class differences in reversal spatial learning and memory performance between adults and first-year chickadees. However, we detected no significant differences in performance in the reversal task between the subset of first-year birds that tested in 2016–2017 and survived until 2017–2018 and the subset that presumably died. It is unclear why we detected age-class differences, but not differences in survival based on reversal learning performance. One explanation is that chickadees improve performance with age, but there were no significant differences in performance in the same cohort of birds over the two years. Age class comparison is based on random sampling of different age classes and expected to be independent of survival detection accuracy. Considering that > 95% of all birds detected around our arrays (both visually and via RFID feeders) are PIT-tagged and almost all detected PIT-tagged birds come to our RFID feeders, we do not think there was a strong bias in our sampling. Nonetheless, it would be important to document direct fitness consequences of variation in reversal learning performance. It is not entirely clear how reversal learning ability is associated with fitness. Reversal learning is a measure of learning flexibility [25–27], so it is not likely involved in food caching and retrieval processes. However, learning flexibility is likely important for foraging in unpredictable and changing environments [22, 23, 25].

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

Our data also suggest that it is unlikely that social/behavioral factors contributed to the observed differences. Juveniles are socially subordinate to adults [28, 29], therefore, if social interactions were an important factor, we would expect an improvement in performance with age. We, however, detected no significant differences in performance in the same individuals first tested as juveniles and then the next year as adults suggesting that our measured performance represents an individual's cognitive ability.

When environmental conditions favor particular heritable traits, natural selection can be expected to result in changes in their frequency distribution within a population, potentially leading to reduced variation and even fixation of a trait [24]. Such results, however, can only be expected in populations lacking new sources of variation (without immigration). In continuously distributed species with no geographic barriers, natural selection across a heterogeneous environment may be essential to maintaining local adaptations in the presence of gene flow [30–34]. In our chickadee system, we previously detected significant differences in spatial cognition and hippocampus morphology [9], yet we found no evidence for population genetic structure suggesting gene flow across elevations [20].

There is large annual variation in severity and longevity of winter conditions at our study site, therefore, strength of natural selection can be expected to vary among years [e.g., 35]. Years with milder, drier winters may be associated with relaxed selection on cognitive ability and, in combination with ongoing gene flow, may result in increased variation in spatial cognition at higher elevations. Alternatively, years with severe winters, such as the 2016–17 season which had the highest snow levels in almost 100 years, may be expected to reduce such variation via natural selection [e.g., 35].

Several other studies investigated fitness consequences of individual variation in cognitive abilities, but focused on novel problem-solving and reproduction [36, 37]. It is not entirely clear how novel problem-solving ability is associated with fitness, whether it is heritable or whether there are population-level differences. We, however, measured spatial learning and memory, which are well known to be involved in cache retrieval [2, 6, 7] and previously documented population differences in spatial learning and memory associated with variation in winter climate [2, 9, 10]. In addition, there is some evidence that spatial learning and memory ability is heritable [13, 15, 18, 19].

To our knowledge, this is the first study providing evidence for natural selection on spatial cognition. Our results support our previous comparative studies showing elevation-related differences in memory and hippocampus morphology over a small spatial scale and suggest that such differences represent local adaptations maintained by natural selection.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENTS AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
 - Study subjects and site
- METHOD DETAILS
 - ‘Smart’ feeders and spatial arrays
 - Spatial learning task
 - Reversal learning task
- QUANTIFICATION AND STATISTICAL ANALYSIS
 - Measuring survival
 - Analyses
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, one table, and two data files and can be found with this article online at <https://doi.org/10.1016/j.cub.2019.01.006>.

A video abstract is available at <https://doi.org/10.1016/j.cub.2019.01.006#mmc5>.

ACKNOWLEDGMENTS

Research presented in this paper was funded by the National Science Foundation (NSF) grants IOS1351295 to V.V.P. and DBI 1556313 to E.B.; Carrie L. Branch was supported by the NSF DDIG 1600845. We are grateful to Jeff Brown and Dan Sayer from Sagehen Field Station (UC Berkeley) for technical assistance after strong snow storms. Maria Tello-Ramos and Dovid Kozlovsky were involved in data collection and analyses during the 2016–2017 year (Tello-Ramos et al., 2018). We thank Josh Firth, Neeltje Boogert, and an anonymous reviewer for thorough and critical comments on our manuscript that resulted in significant improvements. We also thank Felicity Muth and Jake Francis for helpful discussions. We thank Hannah Lansverk for help with graphical abstract.

AUTHOR CONTRIBUTIONS

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

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: November 2, 2018

Revised: December 5, 2018

Accepted: January 2, 2019

Published: February 7, 2019

REFERENCES

- Shettleworth, S.J. (2010). *Cognition, evolution and behavior*, Second Edition (New York: Oxford University Press).
- Pravosudov, V.V., and Roth, T.C. (2013). Cognitive ecology of food hoarding: The evolution of spatial memory and the hippocampus. *Annu. Rev. Ecol. Evol. Syst.* *44*, 173–193.
- Morand-Ferron, J. (2017). Why learn? The adaptive value of associative learning in wild populations. *Curr. Opin. Behav. Sci.* *16*, 73–79.
- Morand-Ferron, J., and Quinn, J.L. (2015). The evolution of cognition in natural populations. *Trends Cogn. Sci.* *19*, 235–237.
- Morand-Ferron, J., Cole, E.F., and Quinn, J.L. (2016). Studying the evolutionary ecology of cognition in the wild: a review of practical and conceptual challenges. *Biol. Rev. Camb. Philos. Soc.* *91*, 367–389.
- Sherry, D.F., Vaccarino, A.L., Buckenham, K., and Herz, R.S. (1989). The hippocampal complex of food-storing birds. *Brain Behav. Evol.* *34*, 308–317.
- Krebs, J.R., Sherry, D.F., Healy, S.D., Perry, V.H., and Vaccarino, A.L. (1989). Hippocampal specialization of food-storing birds. *Proc. Natl. Acad. Sci. USA* *86*, 1388–1392.
- Sherry, D.F. (2006). Neuroecology. *Annu. Rev. Psychol.* *57*, 167–197.
- Freas, C.A., LaDage, L.D., Roth, T.C., and Pravosudov, V.V. (2012). Elevation-related differences in memory and the hippocampus in mountain chickadees, *Poecile gambeli*. *Anim. Behav.* *84*, 121–127.
- Freas, C.A., Bingman, K., Ladage, L.D., and Pravosudov, V.V. (2013). Untangling elevation-related differences in the hippocampus in food-caching mountain chickadees: the effect of a uniform captive environment. *Brain Behav. Evol.* *82*, 199–209.
- Pravosudov, V.V., and Clayton, N.S. (2002). A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behav. Neurosci.* *116*, 515–522.
- Roth, T.C., II, and Pravosudov, V.V. (2009). Hippocampal volumes and neuron numbers increase along a gradient of environmental harshness: a large-scale comparison. *Proc. Biol. Sci.* *276*, 401–405.
- Roth, T.C., II, LaDage, L.D., and Pravosudov, V.V. (2010). Learning capabilities enhanced in harsh environments: a common garden approach. *Proc. Biol. Sci.* *277*, 3187–3193.
- Roth, T.C., 2nd, LaDage, L.D., and Pravosudov, V.V. (2011). Variation in hippocampal morphology along an environmental gradient: controlling for the effects of day length. *Proc. Biol. Sci.* *278*, 2662–2667.
- Roth, T.C., 2nd, LaDage, L.D., Freas, C.A., and Pravosudov, V.V. (2012). Variation in memory and the hippocampus across populations from different climates: a common garden approach. *Proc. Biol. Sci.* *279*, 402–410.
- Chancellor, L.V., Roth, T.C., II, LaDage, L.D., and Pravosudov, V.V. (2011). The effect of environmental harshness on neurogenesis: a large-scale comparison. *Dev. Neurobiol.* *71*, 246–252.
- Freas, C., Roth, T.C., II, LaDage, L.D., and Pravosudov, V.V. (2013). Hippocampal neuron soma size is associated with population differences in winter climate severity in food-caching chickadees. *Funct. Ecol.* *27*, 1341–1349.
- Pravosudov, V.V., Roth, T.C., 2nd, Forister, M.L., Ladage, L.D., Kramer, R., Schilkey, F., and van der Linden, A.M. (2013). Differential hippocampal gene expression is associated with climate-related natural variation in memory and the hippocampus in food-caching chickadees. *Mol. Ecol.* *22*, 397–408.
- Croston, R., Branch, C.L., Kozlovsky, D.Y., Dukas, R., and Pravosudov, V.V. (2015). Heritability and the evolution of cognitive traits. *Behav. Ecol.* *26*, 1447–1459.
- Branch, C.L., Jahner, J.P., Kozlovsky, D.Y., Parchman, T.L., and Pravosudov, V.V. (2017). Absence of population structure across elevational gradients despite large phenotypic variation in mountain chickadees (*Poecile gambeli*). *R. Soc. Open Sci.* *4*, 170057.
- Croston, R., Kozlovsky, D.Y., Branch, C.L., Parchman, T.L., Bridge, E.S., and Pravosudov, V.V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Anim. Behav.* *111*, 225–234.
- Croston, R., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., Bridge, E.S., Parchman, T.L., and Pravosudov, V.V. (2017). Predictably harsh environment is associated with reduced cognitive flexibility in wild food-caching mountain chickadees. *Anim. Behav.* *123*, 139–149.
- Tello-Ramos, M.C., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., Bridge, E.S., and Pravosudov, V.V. (2018). Memory in wild mountain chickadees from different elevations: comparing first year birds with older survivors. *Anim. Behav.* *137*, 149–160.
- Endler, J.A. (1986). *Natural selection in the wild* (Princeton, NJ: Princeton University Press).
- Tello-Ramos, M.C., Branch, C.L., Pitera, A.M., Kozlovsky, D.Y., and Pravosudov, V.V. (2019). Spatial memory and cognitive flexibility trade-offs: to be or not to be flexible, that is the question. *Anim. Behav.* *147*, 129–136.
- Anderson, M.C., and Neely, J.H. (1996). Interference and inhibition in memory retrieval. In *Memory. Handbook of perception and cognition*, E.L. Bjork, and R.A. Bjork, eds. (San Diego, CA: Academic Press), pp. 237–332.
- Strang, C.G., and Sherry, D.F. (2014). Serial reversal learning in bumblebees (*Bombus impatiens*). *Anim. Cogn.* *17*, 723–734.

28. Ekman, J. (1989). Ecology of non-breeding social systems of parus. *Wilson Bull.* 101, 263–288.
29. Mccallum, D.A., Grundel, R., and Dahlsten, D.L. (1999). Mountain Chickadee (*Poecile gambeli*), *The Birds of North America Online*, A. Poole, ed. (Ithaca: Cornell Lab of Ornithology).
30. Gonzalo-Turpin, H., and Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *J. Ecol.* 97, 742–751.
31. McCracken, K.G., Bulgarella, M., Johnson, K.P., Kuhner, M.K., Trucco, J., Valqui, T.H., Wilson, R.E., and Peters, J.L. (2009). Gene flow in the face of countervailing selection: adaptation to high-altitude hypoxia in the betaA hemoglobin subunit of yellow-billed pintails in the Andes. *Mol. Biol. Evol.* 26, 815–827.
32. de León, L.F., Bermingham, E., Podos, J., and Hendry, A.P. (2010). Divergence with gene flow as facilitated by ecological differences: within-island variation in Darwin's finches. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365, 1041–1052.
33. Oliveira, E.F., Gehara, M., São-Pedro, V.A., Chen, X., Myers, E.A., Burbrink, F.T., Mesquita, D.O., Garda, A.A., Colli, G.R., Rodrigues, M.T., et al. (2015). Speciation with gene flow in whiptail lizards from a Neotropical xeric biome. *Mol. Ecol.* 24, 5957–5975.
34. Tigano, A., and Friesen, V.L. (2016). Genomics of local adaptation with gene flow. *Mol. Ecol.* 25, 2144–2164.
35. Kozlovsky, D.Y., Branch, C.L., Pitera, A.M., and Pravosudov, V.V. (2018). Fluctuations in annual climatic extremes are associated with reproductive variation in resident mountain chickadees. *R. Soc. Open Sci.* 5, 171604.
36. Cole, E.F., Morand-Ferron, J., Hinks, A.E., and Quinn, J.L. (2012). Cognitive ability influences reproductive life history variation in the wild. *Curr. Biol.* 22, 1808–1812.
37. Cauchard, L., Boogert, N.J., Lefebvre, L., Dubois, F., and Doligez, B. (2013). Problem-solving performance is correlated with reproductive success in a wild bird population. *Anim. Behav.* 85, 19–26.
38. Bridge, E.S., and Bonter, D.N. (2011). A low-cost radio frequency identification device for ornithological research. *J. Field Ornithol.* 82, 52–59.
39. Branch, C.L., Kozlovsky, D.Y., Croston, R., Pitera, A., and Pravosudov, V.V. (2016). Mountain chickadees return to their post-natal dispersal settlement following long-term captivity. *Behaviour* 153, 551–567.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Mountain chickadee, <i>Poecile gambeli</i>	wild	N/A

CONTACT FOR REAGENTS AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Vladimir V. Pravosudov (vpravosu@unr.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study subjects and site

We tested spatial cognition performance of wild mountain chickadees (*Poecile gambeli*) fitted with unique PIT-tag identifiers (IB Technology, Leicestershire, U.K). We have been trapping and banding chickadees using mist nets at multiple feeder locations throughout the non-breeding season as well as in nest boxes during breeding since 2014 at our long-term high elevation site (ca. 2400 m; 16, 22-24) in Sagehen Experimental Research Forest, near Truckee, CA, U.S.A. All trapped birds were banded with a unique combination of color bands and a PIT-tag. Due numerous issues, birds at our low elevation site (ca. 1900 m) were not tested in the 2017-2018 season. We used data from Tello-Ramos et al. [23] to assess within individual variation in performance across two testing years. The performance of first-year birds that tested during the 2016-2017 season and were detected in the 2017-2018 season, were compared to birds that tested in the 2016-2017 season, but did not reappear during the 2017-2018 season, and were therefore presumed dead. All testing methods in this study were identical to those in Tello-Ramos et al. [23]. Even though we have been banding chickadees since 2014, only the years included in this study allowed us to compare full cohorts of chickadees with known age.

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

METHOD DETAILS

'Smart' feeders and spatial arrays

All spatial memory tasks took place at two different feeder arrays separated by ca. 1.2 km, at the same high elevation locations as in previous studies [9, 10, 21-23]. Each spatial array consisted of eight identical RFID-enabled smart feeders mounted on a square aluminum square frame (122 × 122 cm) equidistant from each other with two feeders on each side. Both arrays were suspended from the four nearby trees up to 4 m above the ground to prevent access by bears and rodents. Both arrays were surrounded by trees on all sides and we showed no significant differences in performance between the arrays [21]. Arrays were only lowered to refill the feeders with seed, collect data, and replace batteries. Each feeder was constructed from PVC panels that housed both a hopper, which contained the black oil sunflower seeds, and a bottom compartment that held RFID circuitry, a lithium-ion battery (12 V, 6800 mAh), and a motor that raised and lowered a rectangular door to restrict and allow access to seeds. An antenna connected to the RFID board was imbedded into a perch in front of the feeder opening. The feeders and antennae were custom made according to the designs by E.S.B and V.V.P [21-23], and the RFID electronics followed the design in Bridge and Bonter [38]. Feeders were programmed to be active from 0600 hours to 1930 hours and when active, feeders recorded PIT-tag IDs, date, and time of visits from all PIT-tagged birds that landed on the perch. Feeder batteries were changed every third or fourth day during testing.

During testing, feeders operated in one of the three feeding modes: open, all, or target. Feeders recorded all visits by any PIT-tagged bird, regardless of the feeding mode in which it was operating. During the pre-testing phase, the feeders were first kept in 'open' mode, where feeder doors remained in the open position and all birds were allowed access to all feeders. Feeders were then set to 'all' mode for one week, in which feeder doors were closed but would open when any PIT-tagged bird landed on the antenna (perch). This allowed birds to habituate to the opening and closing mechanism of the feeder doors. 'Target' mode was initiated at the start of memory testing, where each feeder would only open for birds whose PIT-tag IDs matched a list of IDs that were programmed into the RFID circuit board before testing (different lists of IDs for each feeder). In 'target' mode, all PIT-tagged birds

were recorded at all feeders regardless of feeder assignment to track the order and frequency of visits to unrewarding feeders (location errors) before visiting the rewarding (assigned) feeder. We measured spatial learning and memory by using the number of unrewarding (or unassigned) feeders visited prior to visiting the assigned feeder that provided food. The data were divided into trials, wherein each trial began when a bird visited any feeder in the array and ended with a visit to the rewarding feeder [21–23]. As birds learn, they are expected to make fewer and fewer location errors with each trial and so fewer mean number of location errors per trial averaged over multiple trials is indicative of better learning and memory. Using this system, we previously demonstrated that chickadees learn their assigned spatial feeder by showing a significant reduction in the mean number of location errors per trial with the number of trials [21–23] and significantly lower mean number of location errors per trial averaged over the entire task compared to the expected number of errors based on random search (4 errors). Our previous work suggested that using mean number of location errors per trial over the entire task controlled statistically for the total number of trials is a very accurate and reliable measure of learning and memory performance [21–23]. We have also previously shown that chickadees use spatial learning to identify feeder locations, as they return to their previously assigned location, rather than to a specific feeder after the array has been rotated in space [21].

Spatial learning task

For the spatial learning task, which directly followed the pretesting phase, all feeders were set to ‘target’ mode from 30 March, 2018 to 4 April, 2018. Each bird was assigned only a single feeder (‘target’ feeder) within an array. Assignments were made by first inspecting feeder visit patterns during the pre-testing period, and for each bird visiting the arrays, we assigned the least frequently visited feeder. We also adjusted assignments to ensure that surviving individuals were assigned to different feeders than their assignments in previous years, and that birds that were trapped together were assigned to different feeders.

Reversal learning task

Immediately following the spatial learning task, we conducted a reversal spatial learning task from 4 April to 9 April, 2018, which tests for cognitive flexibility. During the reversal task each bird was re-assigned to a different ‘target’ feeder. All birds previously assigned to the same ‘target’ feeder in the initial learning task, were reassigned to different feeders to avoid social learning. In addition, new, re-assigned feeders were chosen so that they were on a different side of the array than the previously assigned target feeder (detailed methods in 22, 23). Chickadees were expected to stop visiting previously rewarded feeders and learn the location of their newly assigned feeder [23].

QUANTIFICATION AND STATISTICAL ANALYSIS

Measuring survival

All food-caching Parids, such as mountain chickadees, are permanent residents that have one post-natal dispersal movement during late summer-early fall in their first year of life [28, 29]. Such sedentary behavior supports the assumption that any birds banded after their settlement, but not detected later have died, which has been used in all studies of *Poecile* species. In our study system, we have banded more than 1000 chickadees (ca. 500 birds at high elevation, ca. 600 at low elevation) with unique PIT-tags during annual trapping since 2014. Recaptures during annual trapping, combined with detection of PIT-tagged birds at our RFID feeders, were used to detect surviving birds (> 95% annual detection rate of living birds). We have never detected an individual moving from post-natal settlement locations; individuals either continue to be detected around the same trapping/breeding sites or disappear completely. Finally, we showed that birds trapped for behavioral lab experiments in the fall and released after spending months in captivity, returned to their original trapping locations [39]. Therefore, our data are consistent with the general assumption that any chickadee disappearing from our monitored sites between years is likely deceased.

Analyses

For all analyses of spatial cognitive performance, we used the mean number of location errors per trial over the entire learning task and the mean number of location errors per trial made during the first 20 trials of each learning task as dependent variables. Mean number of location errors per trial during the first 20 trials represents initial learning phase that involves learning and short-term memory [21–23]. The overall task performance was defined by the mean number of location errors per trial over the entire task, which likely relies on longer-term memory since birds need to use memory to find the feeder once they learned its location. Smaller mean number of location errors per trial indicate fewer errors on average over multiple trials during the entire 4-day task. We chose the first 20 trials to analyze the initial learning and memory phase because our previous data showed significant differences between birds using this metric [21–23]. The overall task performance over the entire task is more reflective of a longer-term memory and hence represents a different, albeit complimentary information.

We used the total number of trials completed during each task as a covariate to control for potential individual differences in the number and timing of trials [22, 23] and age (adult versus first year) and survival outcome (survived versus died) as independent variables. Total number of trials is negatively associated with the number of location errors per trial due to learning, so using it as a covariate allows direct comparison of performance between groups of interest independently of any potential differences in the number of trials completed [21–23]. We used general linear models and logistic regressions for statistical analyses in Statistica v. 13 (TIBCO Software Inc.). Inclusion of an array as a random variable had no effect on the results and so it was dropped from the analyses.

In all tests, n is the number of birds tested and Figures present either raw data or means and SEM. Significance was defined as P value < 0.05 . We estimated effect sizes for significant results using Glass's $\Delta = (\text{mean } 1 - \text{mean } 2)/SD_2$. See [Table S1](#) for sample sizes.

All of the statistical details of experiments can be found in Results and in the figure legend of [Figure 3](#).

DATA AND SOFTWARE AVAILABILITY

All data from this study are available as supplemental data files included with this paper ([Data S1](#), [Data S2](#)).