Individual variation in spatial memory performance in wild mountain chickadees
from different elevations

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1 ABSTRACT

2 For food caching animals inhabiting environments with strong seasonal variation,
3 harsh winter conditions may limit access to naturally available food and favor the
4 evolution of enhanced spatial memory. Spatial memory enables animals to
5 remember the locations of food caches for overwinter survival, therefore animals in
6 harsher conditions may benefit more from more accurate spatial memory than
7 conspecifics living under relatively mild conditions. Despite numerous laboratory
8 studies lending indirect support to the hypothesis that a harsh environment favors
9 the evolution of spatial memory, there is no direct evidence showing fitness
10 consequences of variation in spatial memory. As a step toward evaluating this
11 hypothesis in natural populations, we established spatial arrays of programmable
12 bird feeders equipped with radio-frequency identification technology (RFID) to test
13 for individual variation in spatial memory in food-caching mountain chickadees
14 (*Poecile gambeli*) at two elevations differing in winter climate severity. Individuals
15 could only access food at a single rewarding feeder within an array of eight, and
16 each individual had to learn the location of their unique rewarding feeder. Birds at
17 higher elevations visited the arrays significantly more frequently than birds at lower
18 elevations, suggesting more intense food caching. They also performed better at
19 locating their rewarding feeder than birds from lower elevations. Individuals
20 showing better performance participated in more overall trials, suggesting a link
21 between food caching propensity and memory performance, but higher levels of
22 activity within each array yielded less accurate memory performance. Finally,
23 rotating the arrays showed that birds relied specifically on spatial memory in order

to locate their rewarding feeder. To our knowledge, this is the first explicit test of spatial memory performance in food caching birds under natural conditions. Our results corroborate earlier laboratory-based work showing large individual variation in spatial memory performance, and lay the groundwork for future investigation into the fitness consequences of individual variation, and the evolution of spatial memory.

KEYWORDS

adaptive specialization hypothesis, mountain chickadee, elevation gradient, food caching, RFID, spatial memory

35 INTRODUCTION

36 Variation in environmental conditions has long been hypothesized to impact
37 the evolution of animal cognition (Dukas, 1998; Shettleworth, 1998), and, in
38 particular, to drive the evolution of specific modular cognitive abilities in food-
39 caching species (e.g. spatial memory, Krebs, 1990; Sherry, 2006). Animals living
40 under seasonally variable conditions are often subject to variation in availability
41 and abundance of food. In general, colder seasons are associated with harsher
42 conditions, including decreased temperatures and increased snow cover, which may
43 limit access to naturally available food sources. Storing food is one strategy that
44 animals may use to cope with this uncertainty (i.e. food caching; Vander Wall, 1990).
45 Scatter-hoarding species store large amounts of food across many different
46 locations during periods of relative abundance (e.g. autumn), which they may then
47 retrieve as needed in times of relative scarcity (e.g. winter). As food-caching
48 animals rely on their caches for overwinter survival (Krebs, Sherry, Healy, Perry, &
49 Vaccarino, 1989; Sherry, Vaccarino, Buckenham, & Herz, 1989; Vander Wall, 1990;
50 Pravosudov & Roth, 2013), spatial memory may be critical for survival in these
51 seasonally fluctuating environments.

52 Cache retrieval involves the use of spatial memory (Krebs et al., 1989; Sherry
53 et al., 1989; Sherry, 2005). Therefore, it can be hypothesized that food caching as an
54 adaptation to unpredictable foraging success makes specific demands on spatial
55 memory and its neural mechanisms (Krebs et al., 1989; Pravosudov & Roth, 2013;
56 Sherry et al., 1989; Shettleworth, 1998; Shettleworth, 2009). Spatial memory is an
57 important component of cognition, defined here as the collection, retention, and use

of information about the environment (Dukas, 2004; Shettleworth, 1998), which specifically involves the use of geographic information and even basic geometry (Kamil & Jones, 1997) in evaluating the relationship between given locations. Evolutionary theory predicts that under conditions of particular climatic harshness (e.g. low ambient temperatures and lengthy seasonal snow cover), the spatial memory ability of year-round resident species may be under strong selection (Krebs et al., 1989; Pravosudov & Clayton, 2002; Pravosudov & Roth, 2013; Sherry, Vaccarino, Buckenham, & Herz, 1989). As a result, it has been predicted that even within species, animals living under harsher climate conditions should have better accuracy and retention of spatial memory than animals living under less harsh conditions (i.e. resulting in adaptive specialization; Pravosudov & Clayton, 2002; Pravosudov & Roth, 2013). This pattern may be predicted across gradients of latitude and longitude (Roth & Pravosudov, 2009), as well as across elevational gradients in the mountains, where dramatic changes in climatic conditions can take place across a relatively small geographic scale (e.g. Freas, LaDage, Roth, & Pravosudov, 2012).

We studied spatial learning and memory in mountain chickadees (*Poecile gambeli*), year-round residents, which inhabit variably harsh conditions along an elevational gradient ranging from approximately 1900 m to 2400 m in elevation in the Sierra Nevada. Relative to birds living at “low” (i.e. 1900 m) elevation conditions, birds at “high” (i.e. 2400 m) elevation conditions within our study site are subject to lower ambient temperatures and increased snow cover, which remains for longer across seasons, resulting in relatively limited access to natural food sources (e.g.

insects and pine seeds) during the winter. Because of this, mountain chickadees inhabiting higher elevations may be selected for increased accuracy, capacity, and/or longevity in spatial memory relative to conspecifics inhabiting lower elevations (Freas et al., 2012). Climate-based variation in spatial memory and neural morphology has been demonstrated in a laboratory setting both in black-capped chickadees (*P. atricapillus*) across latitudinal and longitudinal gradients (Pravosudov & Clayton, 2002; Roth, LaDage, Freas, & Pravosudov, 2011; Roth & Pravosudov, 2009), and in mountain chickadees collected at our specific study sites (Freas et al., 2012; Freas, Bingman, LaDage, & Pravosudov, 2013a; Freas, Roth, LaDage, & Pravosudov, 2013b). In particular, mountain chickadees from high elevations exhibit higher caching propensity, more accurate cache retrieval, increased retention of memory for cache location, and larger hippocampi with more neurons and faster neurogenesis than do their conspecifics from low elevation under laboratory conditions (Freas et al., 2012).

While most laboratory studies support the hypothesis that harsh environments favor the evolution of better spatial memory and associated hippocampal morphology via natural selection (Croston, Branch, Kozlovsky, Roth, LaDage et al., 2015; Pravosudov & Roth, 2013 for review), all of the existing support has been indirect. To establish that natural selection (as local adaptation) accounts for differences in memory and brain anatomy in different environments, it is necessary to demonstrate that heritable individual variation in spatial memory is associated with differential fitness in those environments (e.g. Croston et al., 2015). Understanding whether natural selection is involved in shaping differences in

memory requires examination of cognitive phenotype in the wild, in order to accurately connect variation in cognition with variation in fitness (Endler, 1986). To our knowledge, there have been no studies testing predictions associated with the adaptive specialization hypothesis (e.g. Krebs, 1990) for spatial memory ability in free-living food caching animals.

In this study, we employed RFID (Radio Frequency Identification) technology to directly investigate variation in spatial learning and memory among mountain chickadees residing at two different elevations used in our previous studies (e.g. Freas et al., 2012). We designed and built spatial arrays each consisting of eight “smart” RFID feeders, and implemented these in the field to test how readily resident mountain chickadees learn the correct spatial location of an individually designated target reward feeder. We tested for individual variation in memory performance, based on the prediction that high-elevation birds would perform more accurately than their conspecifics at low elevation. We also tested for effects of bird activity at the array and experience on task performance, and attempted to disentangle the roles of spatial versus local cues in driving the observed performance.

METHODS

Study subjects and site

We trapped and banded mountain chickadees using mist-nets at 17 established feeder sites throughout Sagehen Experimental Forest, near Truckee, CA, USA in the Sierra Nevada mountain range. Birds were trapped at two different elevations: “low”

elevation (approx.. 1900 m) and “high” elevation (approx.. 2400 m) following our previous work (e.g. Branch & Pravosudov, 2015; Freas et al. 2012, 2013a, 2013b; Kozlovsky, Branch, Freas, & Pravosudov, 2014) and fitted with unique color band combinations, including a plastic band containing a passive integrated transponder (PIT) tag (IB Technology; <http://www.buyrfid.co.uk/store/index.php?route=product/category&path=42>).

For each bird, we recorded mass and wing chord length, and we sampled 100 μ L of blood from the brachial vein as conditions allowed (we did not carry out blood sampling in especially cold conditions due to concerns for the welfare of the birds).

Sagehen Experimental Forest is a primarily mixed-conifer forest. With increasing elevation at this site, resident animals face lower ambient temperatures, and increased snow coverage, resulting in harsher and less predictable conditions than at lower elevations. “Smart” feeder spatial arrays were constructed at four previously established feeder locations within our study site, two each at high and low elevation localities, used in our previous work (Branch & Pravosudov, 2015; Branch, Kozlovsky, & Pravosudov, 2015; Freas et al., 2012; Kozlovsky et al., 2014, Kozlovsky, Branch, & Pravosudov, 2015). The distance between two arrays within each elevation was approx. 1.2 km, and arrays drew entirely non-overlapping sets of birds.

“Smart” feeders and spatial arrays

We investigated spatial memory performance based on data from four spatial arrays, each consisting of eight RFID-programmable “smart” feeders mounted on a

square 122 x 122 cm wooden frame (Fig. 1). Within each array, feeders were arranged with two feeders on each side, spaced evenly throughout (approx. 28 cm between adjacent feeders) and with the openings facing outward (Fig. 1). Arrays were suspended from trees approximately 2.5 m. above ground level to prevent access by rodents and black bears (*Ursus americanus*). Feeders were custom designed and manufactured for this study based on design specification by ESB and VVP, using the RFID design described in Bridge and Bonter (2011).

The RFID circuitry, as well as the data-logging and basic motor control, are contained on a single printed circuit board that is similar to that described by Bridge and Bonter (2011). The main operational components of the reader are an RFID module (UB22270, Atmel Corporation, San Jose, CA), microprocessor (PIC16F688, Microchip Inc., Chandler, AZ), memory module (24LC512, Microchip Inc.), and real-time clock (DS1307, Maxim Integrated Products, Sunnyvale, CA). The main differences include the incorporation of an SD card slot that allows for removable data storage and improved capacity to support external devices like motors and sensors. The circuit board powered a carrier signal emitted by a loop antenna mounted in a wooden perch on the front of the feeder (Fig. 1). The carrier wave would then energize passive RFID tags that came in range and induce them to emit a unique 10-ascii character code, which the reader received and recorded along with a time stamp. Motor control was implemented on the same processor that orchestrated RFID function and data logging. However, it was necessary to incorporate an external transistor circuit to switch current to the motor on and off. The motor was attached directly to a non-transparent plexiglass wheel with holes

that could restrict or allow food access. The position of the wheel was monitored by a small snap-action switch that could close or open a circuit when its sensor arm was pressed or released by tabs on the edge of the rotating wheel. The switch was connected directly to an I/O port on the microprocessor such that power to the motor could be turned on or off in response to the position of the rotating plate. Motion in the motor and plate was triggered by the presence of a tagged bird perched on the antenna, which consisted of wire coiled tightly around a notched wooden block. Antenna wires were wrapped within the notches of identically built and sloped wooden blocks to ensure similar performance. Each antenna was tested before installation and the inductance measured between 1.345 and 1.355 mH (an inductance value of 1.350 mH provides optimal RFID detection ; <http://animalmigration.org/RFID/RFIDbuild.htm>). The wire was subsequently encased in epoxy to prevent movement and misshaping and to protect from ambient moisture. Correct and consistent operation of the feeders, including antennae, was verified every second day with at least two different RFID tags throughout testing.

The feeder system allowed for three modes of operation, which provided variable access to sunflower seed housed within the feeder body. In the “open” mode, the access door remained open. Any bird could land on the perch and take food, and all visits by birds with RFID tags were recorded. The second mode, “feed all”, would open the feeder to any bird with an RFID tag and record the tag ID and time stamp, but birds with no RFID band would not be able to obtain food. Finally, the “target” mode would only open the feeder for birds whose unique RFID codes were contained in a list of ID numbers entered manually into the microprocessor

memory – these birds were assigned to that particular feeder target. The RFID reader recorded all visits to the perch regardless of whether or not the feeder opened.

Feeders cycled on an automatic timer, and were active from 0600 hours to 1930 hours, during which time they operated on a 50% duty cycle, polling for tags for 0.5 s and then shifting into a low-power mode for 0.5 s. Feeders were filled with hulled sunflower seeds, which could only be accessed through a hole in the rotating wheel described above.

Memory testing

To acclimate the birds to the feeders, they were first presented with feeders in the “open” mode for approximately 30 days. Beginning 12 January 2015, we initiated a two-week training phase wherein all feeders were programmed to the “feed all” mode, to allow the birds to habituate to the feeder mechanism and movement. The memory testing phase began on 26 January 2015, and lasted for 19 days. During this phase the feeders were switched to “target” mode, and birds were able to obtain food from only a single, randomly assigned “target” feeder. Targets were assigned to individuals at random from the subset of individuals known to frequent each feeder array (from pilot data), and each individual was assigned only one target feeder. Upon a visit to the correct target feeder, the plexiglass wheel rotated and feeders moved to the open position. Feeders remained open until the individual flew from the perch, after which they moved to the closed position until the antenna again recorded the ID of a target individual assigned to that feeder.

Assigning different individuals to different target feeders within each array should largely prevent social learning, as following a conspecific to their rewarding feeder is highly unlikely to result in a reward. Hence this paradigm should discourage birds from following each other, and instead reward the birds only for learning their own target feeders.

To test memory, we recorded all instances of any individual landing at any feeder, and subsequently matched each bird to its target feeder and compiled both the number of visits to non-target feeders (“visit errors”) per trial per individual, and the number of incorrect feeders visited (“location errors”) per trial per individual. A “visit error”, then, is an instance of landing on the perch of any incorrect feeder, even when the same individual lands on the same incorrect feeder multiple times within a trial, whereas a “location error” is an incorrect feeder that is visited. A bird might, for example, make two location errors if it visits two different incorrect feeders within a single trial, but if in that same trial it visits these two feeders multiple times, the number of visit errors will be larger. A trial was defined as beginning upon the first visit to an array either 1) each day, or 2) since the most recent correct choice visit, and ending with either 1) a visit to the target feeder, or 2) the end of the day.

Control test – do birds use spatial memory?

Immediately following the memory-testing phase, we conducted a second, one-day experiment to determine whether the birds used spatial memory to locate the correct feeders or whether they relied on other, local cues (i.e. subtle differences

among individual feeders, which are impossible to avoid). It is generally assumed that food-caching birds use spatial cues primarily, even when both spatial and non-spatial cues are present (e.g. Brodbeck, 1994). This assumption has, however, never been tested in wild birds under natural conditions. At the end of the first testing phase, we rotated each array 180° in orientation, and reprogrammed the feeders such that every individual was assigned to the feeder now located in the same spatial position within the array as was previously rewarded (the individual's "target location"). We allowed the birds to visit the feeders as before, and recorded each instance of each individual landing at any feeder until the end of that individual's first trial. The first trial ended either when an individual received a reward, or at the end of the control testing day. We then calculated the number of location errors (incorrect feeders visited per trial) relative to both the previous location of the target feeder ("target location") and the previously assigned feeder in its new location ("target feeder"). We regarded visiting the target location prior to visiting the target feeder as evidence of stronger reliance on spatial memory, whereas visiting the target feeder prior to visiting the target location was interpreted as stronger reliance on local cues associated with the appearance of the previously rewarding feeder.

Analysis

Changes in spatial memory performance

We compared the mean daily number of visit errors and the mean daily number of location errors per trial within each individual across high and low

elevation populations using repeated measures Generalized Linear Mixed Models (GLMMs) fit by maximum likelihood with Satterthwaite approximations using R (R Core Team, 2014) package lme4 (Bates, Maechler, Bolker, & Walker, 2014). We fit these models such that they contained both random intercepts and random slopes for each individual RFID number, and allowed for differences in the rate of change in response over time among different individual birds. The mean daily number of visit errors/location errors per bird was treated as the response variable, with RFID as a random variable accounting for unbalanced repeated measures, and elevation (high versus low) and test day (unique to that individual bird's testing window; henceforth "bird day") were included as fixed effects. RFID was implicitly nested within elevation in this model, and bird day was nested with RFID, as this was a repeated measure unique to each individual. Bird day was used to account for time throughout testing because participation in this experiment was voluntary, therefore each bird varied in its number of trials and testing days. Learning is indicated by a decrease in the number of both errors and location errors, as birds should learn to visit only the rewarding feeder.

Differences among elevations

Because variation in the shape of learning curves for individual birds could obscure systematic differences in the overall number of errors and location errors between individuals at high versus low elevations, we averaged data (number of errors and location errors per trial) across each individual's testing window to yield single metrics of performance for each bird. To test for differences in performance

between high and low elevations, we first compared the mean number of location errors across all testing days by using a General Linear Model (GLM) with elevation as a fixed effect and the number of visit errors as a covariate. We then fit a repeated measures Analysis of Variance (ANOVA), where the mean number of location errors per trial for each bird and the mean number of location errors per trial for each bird excluding bird day one were repeated measures (e.g. mean number of location errors per trial across all bird days and mean number of location errors per trial across all bird days except the first day of testing). Here, elevation, mean number of visit errors per trial, and mean number of birds visiting the array per day for each individual's testing window were predictors. Day one of testing was parsed out for this analysis because the nature of the test necessitates that birds initially explore the array in order to identify their correct feeder. Day one of testing is necessarily the most random, which could falsely inflate inter-individual variation and dilute quantifiable effects of differences in learning. At the same time, comparing performance with and without including day one allows to test for potential differences in learning rates. Following this comparison, all further analyses were conducted using only the data excluding the first day of testing

As the number of visit errors and the number of location errors are tightly correlated (see Results), and variation in location errors likely better reflects real variation in memory (e.g. the number of visit errors may disproportionately inflate artifacts of flight path to or from the target feeder, or of limited access to a particular feeder perch when another bird has landed on it), we focus the remaining analyses on memory performance as measured only by location errors. We compared

improvement in performance between elevations by subtracting the mean number of location errors per trial per bird across the entire testing window from the mean number of location errors per trial per bird beginning on bird day 2. We compared these measures across elevations using a GLM.

Relationship between total number of trials and learning

Because we found significant effects of elevation associated with the number of trials an individual completed over the course of testing (see Results below), we also tested for an association between the mean number of location errors per trial per bird, and the total number of trials completed by each bird. The number of completed trials indicates the total number of potential feeding events and, likely, food caching activity. We have previously shown that chickadees from high elevations cache significantly more than chickadees from low elevation in a controlled laboratory environment (Freas et al., 2012). Based on these results, it may be predicted that birds from high elevation should visit the arrays more frequently and complete more trials. By extension, individuals that cache more food and complete more trials may have better spatial memory than individuals that cache less food. Alternatively, participation in more trials might directly affect memory performance. Yet another alternative might be that birds with poorer memory compensate for this by caching more food, in which case we would predict a positive correlation between the number of location errors and the number of completed trials.

In order to disentangle whether 1) completing a higher number of trials resulted in improved performance, or 2) more accurate performers completed more trials, we first fit a linear regression with the mean number of location errors per trial across the entire testing window for each bird as the response, and the total number of trials completed by that individual as the predictor. Next, we fit the same linear regression, only this time using location error data from the second day of testing for each bird only (predictor data were the same as in the previous regression). As birds have completed only a small subset of their total trials on bird day two, a significant positive correlation in this analysis would favor the second aforementioned possibility, while a significant correlation in the first, but not in the second analysis would support the possibility that performance improves across trials simply due to increased experience.

To approach this question a second way, we fit a repeated measures GLMM with location errors as the response variable, the total number of trials as fixed effect, and bird day nested within RFID as a random effect. We fit two versions of this model based on the number of location errors plotted in Fig. 2a. The first model included only the subset of birds completing more than 500 trials in total across their entire testing window, while the second model contained only the subset of birds completing less than 500 trials in total across their entire testing window. If birds that complete more total trials perform more accurately, we would expect no significant relationship between the total number of trials and mean number of location errors per trial for birds that completed more than 500 total trials. Conversely, birds completing fewer than 500 total trials should show a significant

reduction in mean number of location errors per trial with the total number of trials completed. These models allowed us to compare the predictive value of the total number of trials for low- versus high-participating birds.

Effects of bird activity at the array

Because we found significant variation among arrays for the above-described measures of overall level of activity, we next fit separate GLMs describing variation in several metrics of bird activity at the array. We fit two separate models including array as a categorical predictor variable. The first included the mean number of birds visiting the array across each bird's unique testing window as a response variable, while the second included the mean number of total trials completed at each array across each bird's unique testing window as a response variable.

Next, we fit models including the mean daily number of location errors per trial as the response variable, array as an independent categorical variable, and mean number of visits to the array per day across that bird's testing window and mean number birds visiting the array per day across that bird's testing window as covariates. These analyses allowed us to test specifically for effects on location error frequency associated with variation in bird activity at the array during testing, which may account for some of the observed variation in testing performance irrespective of actual memory accuracy.

Control phase

378 To test for the use of spatial versus local cues in locating the targets, we
379 compared the number of location errors for each bird relative to both their target
380 location from the previous testing phase (which contained a new feeder during this
381 test, see Methods above), and the target feeder from the previous testing phase in its
382 new spatial location. For the first trial immediately following array rotation, we
383 compared the number of location errors relative to the correct target location
384 versus the number of location errors relative to the correct target feeder (i.e. the
385 previously rewarding feeder) using a repeated measures GLM. For this analysis, the
386 number of location errors relative to the correct target location and the number of
387 location errors relative to the correct target feeder were treated as repeated
388 measures predicting elevation.

389 Because birds were rewarded after their first visit to the correct target
390 location even during this control trial (but not to the correct target feeder, which
391 provided reward in the earlier round of testing), it was necessary to categorize error
392 comparisons (the number of location errors relative to the target feeder relative to
393 the number of location errors relative to the target location) as 'greater' or 'lesser'
394 rather than compare the direct count of location errors relative to each target
395 directly, in order to avoid false inflation of the metric for number of location errors
396 relative to the correct target feeder. That is, if a bird first visited the correct target
397 location strictly by chance and was rewarded there, it may continue to visit that
398 location based on that instance of reward and never visit any other feeder, despite
399 not having actually learned the spatial association in the earlier testing phase. To
400 test whether the birds visited either the correct target location or the correct target

feeder preferentially, we compared the number of individuals that visited the correct spatial location prior to visiting the previously rewarding feeder using a two-tailed binomial test. Likewise, if birds relied on spatial cues, most birds would be expected to visit the correct spatial location feeder first.

Analyses were conducted in R version 3.1.1 (R Core Team, 2014), and Statistica version 9 (Statsoft, Inc.).

RESULTS

Memory testing phase

In total, we obtained data from 86 individuals ($N = 51$ high elevation, $N = 35$ low elevation). The mean number of errors per trial across all individuals at all four spatial arrays throughout testing was 3.73, and ranged from 0 to 73 errors across the entire testing period. The mean number of location errors per trial across all individuals at all four spatial arrays throughout testing was 1.39, and necessarily ranged from 0 to 7, with a mean of 3.5 errors expected if birds were searching at random (Fig. 3).

Changes in spatial memory performance

The mean number of visit errors per day decreased significantly across days of testing (bird day $t_{63} = -6.82$, $P < 0.001$), but the GLMM revealed no significant differences between elevations (elevation $t_{69} = -0.81$, $P = 0.42$; full model adjusted $R^2 = 0.27$). The model predicting the mean daily number of location errors

corroborated this result (GLMM: bird day $t_{52} = -7.9$, $P < 0.001$; elevation $t_{80} = -0.14$, $P = 0.89$; full model adjusted $R^2 = 0.57$). These analyses indicate a significant reduction in the number of errors over time (Fig. 4), yet we also see large amounts of individual variation in learning (Fig. 4).

Differences among elevations

The mean daily number of visit errors across the entire testing period beginning on bird day two was significantly correlated with the mean daily number of location errors (Adjusted $R^2 = 0.76$, $t_{80} = 16.77$, $P < 0.001$). Birds at low elevation made significantly more location errors relative to the total number of errors (GLM: elevation $F_{1,79} = 5.31$, $P = 0.02$; number of errors as a covariate $F_{1,79} = 292.9$, $P < 0.001$; Full Model adjusted $R^2 = 0.79$; Fig. 5a) than did birds at high elevation.

A repeated measures GLM (Full Model adjusted $R^2 = 0.80$) with the mean daily location errors during the entire testing period including the first day of testing versus the mean daily location errors beginning on bird day two showed no significant differences between the two measures ($F_{1,77} = 0.65$, $P = 0.42$) and no significant effect of elevation ($F_{1,77} = 1.51$, $P = 0.22$). The interaction between elevation and the repeated measure, however, was significant ($F_{1,77} = 4.12$, $P = 0.04$, Fig. 5b), indicating significant differences in the rate of change across the repeated measure between elevations, such that birds at high elevation improved more after the first day than did birds at low elevation. Pairwise differences among all combinations of location errors and elevation (Tukey LSD: pooled MSE = 0.2482, $df = 79.89$, all $P < 0.05$) were significant, except between the two measures from high

elevation ($P = 0.47$). Among the covariates included in the analysis, the number of visit errors ($F_{1,77} = 127.48, P < 0.001$) and the number of trials ($F_{1,77} = 6.01, P < 0.05$) were significantly different, but the mean number of birds visiting the array per day ($F_{1,77} = 2.48, P = 0.12$.) was not. Likewise, in a separate comparison of differences in mean daily location errors spanning all trials across all days of testing and data excluding bird day one across elevations, performance at high elevation improved significantly more than performance at low elevation (GLM: $F_{1,80} = 4.23, P = 0.04$).

Relationship between total number of trials and learning

Birds at high elevation participated in significantly more trials than birds at low elevation (GLM: $F_{1,80} = 18.73, P < 0.001$; Fig. 6), therefore we tested whether the total number of trials was associated with the mean daily number of location errors. The mean number of location errors per trial across the entire testing window for each bird was significantly and negatively associated with the total number of trials an individual completed across the entire testing period (Adjusted $R^2 = 0.31, t = -6.16, P < 0.001$; Fig. 2a). The mean number of location errors per trial on bird day 2 of testing *only* was also significantly and negatively associated with the total number of trials across the entire testing period (Adjusted $R^2 = 0.16, t = -4.11, P < 0.001$, Fig. 2b). Thus, birds which would ultimately perform the most trials made fewer location errors relative to other individuals as early as their second day of testing.

For the subset of individuals completing fewer than 500 trials in total, the total number of trials was a significant predictor of the mean daily number of location errors during the testing period after the first day of testing (GLMM, $t_{52.29} =$

-4.37, $P < 0.001$; full model Adjusted $R^2 = 0.55$). For individuals completing more than 500 trials across their entire testing window, however, the total number of trials did not predict the mean daily number of location errors after the first day of testing (GLMM, $t_{24.55} = -1.71$, $P = 0.1$). Therefore, performance does not seem to improve with participation beyond 500 trials. Fewer than 500 trials were needed to perform accurately on this memory task, yet some birds completed as many as 2000 trials.

Effects of bird activity at the array

The mean number of birds visiting each array per day was not a significant predictor of the number of location errors in the repeated measures GLM, which included number of errors and total number of trials as covariates (see above). However, there were significant differences in the mean number of birds visiting per day across the arrays (GLM: $F_{1,3} = 1381.45$, $P < 0.05$). Likewise, differences across arrays in the number of trials completed per individual were statistically significant (GLM: array $F_{3,78} = 6.59$, $P = 0.001$, Fig. 6). Because of these differences, we tested predictive effects of bird activity (both as number of individuals and as number of visits) on the number of location errors within arrays. The separate models including array as an independent variable in the analysis of the mean daily location errors showed that the mean number of birds present at the array (GLM: array $F_{3,77} = 6.56$, $P < 0.001$; number of birds $F_{1,77} = 11.34$, $P = 0.001$; Full model Adjusted $R^2 = 0.16$) and the total daily number of visits (GLM: array $F_{3,77} = 9.35$, $P < 0.001$; daily visits $F_{1,77} = 19.91$, $P < 0.001$; Full model Adjusted $R^2 = 0.24$) had

significant effects on memory performance. In other words, having more PIT-tagged individuals present at the array was associated with a higher number of location errors.

Control phase

After the arrays were rotated 180°, 73% of all individuals tested visited the correct target location prior to visiting the correct target feeder (the previously rewarding feeder in its new spatial position; Fig. 7). At high elevation, 71% of individuals and at low elevation, 77% of individuals visited the correct location first. Both within high and low elevations, and across both elevations, significantly more individuals visited the correct target location prior to the correct target feeder (binomial test: all $P < 0.05$; Fig. 8).

The number of location errors relative to the correct target feeder was significantly greater than the number of location errors relative to the correct target location (repeated measures ANOVA: $F_{1,61} = 14.24$; $P < 0.001$). This effect did not vary with elevation ($F_{1,61} = 2.59$, $P = 0.1129$), and the interaction was not statistically significant ($F_{1,61} = 0.04$, $P = 0.83$; Fig. 14). Across all arrays, 54% of individuals did not land on the correct target feeder at any point during the control trial.

Ethical note

This research adhered to the guidelines of the Institutional Animal Care and Use Committee of the University of Nevada – Reno (Protocol #00603), as well as all local

and federal guidelines. We did not detect any detrimental effects of tagging and collecting blood samples.

DISCUSSION

We have successfully designed and implemented an RFID-based system to test spatial memory in food-caching birds in their natural environment. Our study showed that mountain chickadees successfully learned the spatial memory task, as indicated by significant improvement in performance across the testing period. Yet, we also showed large individual variation in spatial memory performance. Our results corroborate our previous laboratory findings that chickadees from high elevation have more accurate spatial memory than chickadees from low elevation (Freas et al., 2012), but here we have shown these differences under natural conditions using free-ranging birds. In addition, we found that birds that completed more trials performed more accurately, as early as the second day of testing. Finally, we discovered that a more active array, with more bird “traffic”, is associated with poorer performance, most likely due to social interference.

Chickadees living at higher elevations made fewer location errors on a spatial learning task performed under natural conditions than did their lower elevation counterparts (Fig. 5), despite physical separation of only approximately 10 km in distance. Additionally, birds at high elevation completed significantly more trials. Because of differences in environment at high versus low elevation, the observed differences in performance could be the result of immediate differences in

temperature and snow cover across testing localities. However, previous studies using birds sourced from these locations and brought into a laboratory setting for testing have shown significant differences between high and low elevations in caching propensity, accuracy in cache retrieval, and memory retention, as well as associated differences in hippocampal volume, neuron number, and neurogenesis rates (Freas et al., 2012). Combined with these laboratory results, our field data suggest that the differences we observed both in spatial memory and in food caching (as indicated by a larger number of completed trials) were not simply due to immediate differences in conditions, though conditions at high elevation during our testing were more severe than those at low elevations.

One of the important discoveries in our study was the significant negative association between the number of location errors (i.e. spatial memory performance) and the total number of trials completed across the entire testing window. Birds completing more trials made fewer location errors, showing better memory performance (Fig. 2). This held true even for a subset of data including only each bird's second day of participation, indicating that even when the bulk of trials had not yet been completed, birds that would ultimately complete more trials showed better memory performance. There are two possible explanations for the observation that better spatial memory performance is associated with participation in more total trials: 1) birds that participated in more trials performed well because they had more experience and therefore more opportunities to learn, or 2) birds performing well were also the most motivated to visit their target feeder, resulting in high visit rates among these high performers. It is unlikely that these

birds were immediately consuming each seed upon reward, as this would mean consuming several hundred sunflower seeds per day for many individuals, therefore completing more trials is more likely associated with more intense food caching activity. In our study, chickadees from high elevation participated in significantly more trials than chickadees from low elevation, suggesting that high elevation birds cache more food than their low elevation counterparts. This result is consistent with a previous finding that high elevation birds cache significantly more food, even in controlled and stable laboratory conditions (Freas et al., 2012).

Hypothetically, it is possible that the number of errors per trial could constrain the number of trials by definition, as we define a trial as terminating with a visit to the correct target feeder. However, for this to be the case, individuals must be constrained in their number of visits to the array as a whole by the amount of time available for test participation. In other words, if each individual visits the array at the same rate, yet some birds are more accurate, these birds will have participated in more trials than less accurate individuals by definition. However, as we know that individuals vary widely in timing and propensity of visitation to the array, as well as performance on the memory task, we believe that this is unlikely to have had systematic effect on our data, and cannot explain the relationship between performance and participation.

As high elevation birds in our study completed significantly more trials on average than low elevation birds, and also showed significantly better spatial memory performance, our results also indirectly suggest that individuals with higher caching propensity seem to have better spatial memory. In black-capped

chickadees, food caching propensity and spatial memory ability were linked in a common garden experiment comparing two populations from different environments (Roth et al., 2012). It is possible, then, that there exists a common mechanism linking caching propensity and spatial memory ability in food-caching birds more generally. Further research should investigate this pattern across food-caching species more broadly, while controlling for effects of phylogeny.

Likewise, we found a significant correlation between memory performance and the total number of trials in the subset of individuals completing fewer than 500 trials across their entire testing window (excluding the first day), but for the subset of individuals completing more than 500 trials, this relationship was not significant. Individuals completing fewer than 500 total trials, then, continued to improve with experience after the second day of testing (bird day 2), whereas individuals completing more than 500 total trials ceased to show significant improvement with additional experience after the second day of testing. This suggests that these birds had reached their best performance level already on the second day of testing and that completing hundreds more trials did not result in any further improvement. These results also provide further indirect support for the scenario wherein individuals performing more accurately go on to complete more trials.

Our results have revealed significant variation in performance associated with temporary differences in activity level within arrays. Chickadees that participated in memory testing on days with more social traffic (i.e. more birds, more completed trials, more visits overall), made more location errors than those testing on days with relatively less social activity. In other words, chickadees seem

607 to make more location errors when more birds were present at the arrays and when
608 there was more activity. Because the same data (i.e. mean number of trials per
609 individual) have also shown that individuals completing more trials are more
610 accurate than individuals completing fewer trials, we believe that this trend is
611 unlikely to reflect “true errors”, but rather, performance errors resulting from social
612 interference at the feeders (e.g. displacement, visits to incorrect feeders either
613 following another individual’s being rewarded at their separate target feeder or
614 waiting for the correct feeder to become vacant). Social interactions, particularly
615 dominance interactions, are known to effect cognitive task performance in social
616 species (e.g. Boogert, Reader, & Laland, 2006; Pravosudov, Mendoza, & Clayton,
617 2003), and it is often necessary to control for the presence of other individuals in
618 order to accurately measure learning and memory. In this case, however, as our goal
619 is to measure performance as it may impact fitness in a wild population, the effects
620 of social interactions are an important source of variation rather than “noise”, and
621 further study should be directed toward determining whether interacting effects of,
622 for example, social dominance and spatial memory accuracy may give rise to fitness
623 consequences in wild populations. Interestingly, even though the number of birds
624 and completed trials were different among arrays, such differences were not
625 associated with variation in spatial memory performance. The differences we
626 detected were only associated with the much smaller within-array variation in array
627 “traffic”. While our paradigm specifically discourages social learning because
628 following other individuals would, in most cases, result in failure to obtain food,
629 there may yet remain unaccounted-for differences in individual motivation which

may be partially attributable to differences in foraging group size within an array. Additionally, we could not account for any potential effects of unbanded birds which may have been present at the arrays, though these birds also could not lead to the rewarding feeder. We have, however, no *a priori* reason to predict that these would lead to patterned difference in performance.

Finally, we have provided the first explicit test of the use of spatial cues by food-caching chickadees in a field setting. Laboratory studies suggest that food-caching birds preferentially rely on spatial cues even when both spatial and non-spatial (e.g. local) cues are available (e.g. Brodbeck, 1994), although subsequent work in the laboratory has shown that food-caching species can prefer non-spatial cues when they seem to be simpler than the spatial cues (e.g. LaDage, Roth, Fox, & Pravosudov, 2009). Regardless of whether food-caching species use spatial cues preferentially, it is clear that the use of local cues is available to them as well. By demonstrating that chickadees made fewer errors relative to a spatially cued target than to a locally cued target, we have demonstrated specific demand on spatial memory, which may be under selection among scatter-hoarding animals as a result of ephemeral, unpredictable food supplies. Although the link between spatial memory and fitness is widely assumed in studies investigating the evolution of cognition more broadly and of spatial memory in particular, ours is the first explicit test of spatial memory use in the context in which we expect selection to be taking place.

Superior memory among individuals under harsher climatic conditions may facilitate the retrieval of stored food in periods of relative scarcity, and should lead

to increased fitness. In establishing a field-based system to test spatial memory in a food-caching species and by demonstrating large individual variation in spatial memory performance that relates to environmental conditions, we have built a foundation upon which we can directly assess the fitness consequences of variation in spatial memory in environments with ostensibly different selection pressure on memory (e.g. Freas et al. 2012; Roth & Pravosudov 2013). Our results are consistent with the predictions laid out by the adaptive specialization hypothesis – that relatively harsher climates result in increased demand on caching propensity and spatial memory (Freas et al. 2012; Pravosudov & Clayton, 2002; Roth & Pravosudov, 2009), even on a small spatial scale where climate varies as a product of variation in elevation. Future research, then, should directly test for variation in survivorship and reproduction, and investigate potential links between spatial memory performance and fitness in free-living populations.

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Figure 1. (a) Schematic of spatial array design. Each array consisted of a wooden frame (122 × 122 cm) with eight feeders mounted to the outer edges. Arrays were suspended with wire to hang from surrounding trees, allowing equal access to feeders on all four sides. (b) Photograph of completed ‘smart’ feeder, front view. Wooden block with wire wrapping acts as both a perch and an antenna, where birds can land to access seed from feeder opening when the feeder is in the open position. Metal plate on front face of feeder protects rotating wheel and switch from outside elements, and cap prevents precipitation from entering main feeder compartment.

Figure 2. Mean daily location errors per trial per bird across the entire testing window (circles) and excluding bird day 1 (triangles). Each point represents one individual. Boxes represent the SE and the spread represents the SD of the mean.

Figure 3. Individual variation in spatial memory performance based on the mean daily number of (a) visit errors and (b) location errors per trial across each bird day of testing. Raw data are superimposed on box plots (mean, SE and SD) describing variation for each day, by elevation. Each dot represents one individual on 1 day of testing. Filled points represent individuals from low elevation; open points represent individuals from high elevation.

Figure 4. (a) Mean $\pm$ SE daily number of location errors per trial at high versus low elevation. (b) Least squares means $\pm$ SE from repeated measures GLM of the mean number of location errors per trial per individual across the entire testing period (all trials) and excluding bird day 1 (beginning on bird day 2). Model includes the mean number of birds visiting the array per day across each individual’s testing window, the mean number of errors per trial and the elevation (high versus low).

Figure 5. Mean $\pm$ SE variation in the total number of trials completed per individual at each array across the entire testing period.

Figure 6. (a) Mean daily location errors per trial (excluding data from bird day 1) relative to the total number of trials completed by each bird across its entire testing window. (b) Mean location errors on bird day 2 relative to the total number of trials completed by each bird across its entire testing window.

Figure 7. Percentage of individuals that visited the correct (previously rewarded) feeder prior to visiting the correct reward location ('target feeder first') and that visited the correct reward location prior to visiting the correct reward feeder ('target location first') during the control phase.

Figure 8. Least squares means $\pm$ SE of the number of location errors relative to the correct reward location ('target location') and the correct reward feeder ('target feeder').

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