

# A Test of the Adaptive Specialization Hypothesis: Population Differences in Caching, Memory, and the Hippocampus in Black-Capped Chickadees (*Poecile atricapilla*)

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To test the hypothesis that accurate cache recovery is more critical for birds that live in harsh conditions where the food supply is limited and unpredictable, the authors compared food caching, memory, and the hippocampus of black-capped chickadees (*Poecile atricapilla*) from Alaska and Colorado. Under identical laboratory conditions, Alaska chickadees (a) cached significantly more food; (b) were more efficient at cache recovery; (c) performed more accurately on one-trial associative learning tasks in which birds had to rely on spatial memory, but did not differ when tested on a nonspatial version of this task; and (d) had significantly larger hippocampal volumes containing more neurons compared with Colorado chickadees. The results support the hypothesis that these population differences may reflect adaptations to a harsh environment.

Some species of animals regularly cache food and rely on memory to retrieve their caches at a later time when supplies are less abundant (for a review, see Vander Wall, 1990). A Clark's nutcracker (*Nucifraga columbiana*), for example, may cache about 33,000 seeds a year and remember cache locations for as long as 9 months (Balda & Kamil, 1992). Some boreal parids have been estimated to cache up to 500,000 food items per year (Brodin, 1994; Haftorn, 1956; Pravosudov, 1985). The number of items cached and the length of time they are left before recovery vary from species to species. One reason for this species difference may be that reliance on stored food may be greater for those living in harsher environments, where failure to recover food caches in the winter may result in death from starvation (Pravosudov & Grubb,

1997a; Pravosudov & Lucas, 2001). And in the laboratory, demanding foraging conditions such as limited and unpredictable food supply result in increased caching intensity (Hurly, 1992; McNamara, Houston, & Krebs, 1990; Pravosudov & Grubb, 1997a, 1997b) and more efficient cache recovery, as shown by the number of visits subjects made to find a cache (Pravosudov & Clayton, 2001).

The capacity and longevity of cache location memories have led a number of authors to hypothesize that the increased visuospatial demands of remembering the locations of so many scatter-hoarded food caches are associated with an enlargement of the hippocampus (e.g., Krebs, 1990; Krebs et al., 1996; Shettleworth, 1995; Shettleworth & Hampton, 1998), an area of the brain that is known to play a role in memory, including that required for accurate cache recovery (Sherry & Vaccarino, 1989). According to the adaptive specialization hypothesis, food-caching animals should not only have larger hippocampal volumes (relative to overall brain and body size) than their noncaching counterparts, but they should also outperform noncaching species on tests of spatial memory. In contrast, there should be no difference on nonspatial memory tasks.

Evidence in support of the hypothesis that hippocampal enlargement is an adaptive specialization of food-storing species comes from phylogenetic analyses of hippocampal size across a variety of families and subfamilies of birds (Krebs, Sherry, Healy, Perry, & Vaccarino, 1989; Sherry, Vaccarino, Buckenham, & Hertz, 1989). These studies show that food-caching species do have larger hippocampal volumes relative to the remainder of the telencephalon than do noncaching species. What is less clear is whether food-caching birds also perform better than their noncaching relatives on spatial memory tests. Some studies do provide results that support the adaptive specialization hypothesis (e.g., Balda & Kamil, 1989; Biegler, McGregor, Krebs, & Healy, 2001; Kamil, Balda, & Olson, 1994; Olson, Kamil, Balda, & Nims, 1995), but,

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on balance, the results have been mixed (for a review, see Shettleworth, 1995; see also Bolhuis & Macphail, 2001).

The fact that food-caching species vary in their reliance on stored food over the winter months allows an additional test of the adaptive specialization hypothesis, namely, that those species that cache more intensively should have larger relative hippocampal volumes than those that cache less intensively. Hampton and Sherry (1992) and Hampton, Sherry, Shettleworth, Khurgel, and Ivy (1995) reported that parid species living farther north cache more food and have larger hippocampi compared with species living farther south. Healy and Krebs (1992, 1996) also found a significant positive correlation between hippocampal size and reliance on cached food in European species of corvids and parids.

The relationship is less clear for some of the comparisons within families of North American corvids and woodpeckers (e.g., for hippocampal volume, see Basil, Kamil, Balda, & Fite, 1996; Volman, Grubb, & Schuett, 1997; for reviews of the memory studies, see Macphail & Bolhuis, 2001; Shettleworth, 1995). One possible reason for this is that reliance on stored food is confounded with many other factors such as sociality, territory size, and diet. Efficient cache recovery depends on the birds remembering where they cached their food. In contrast to specialist feeders that cache only seeds, however, species such as the Western scrub jay (*Aphelocoma californica*) that cache both perishable and nonperishable foods may need to keep track of the relative time elapsed since caching, as well as the contents of their caches (Clayton & Dickinson, 1998, 1999a, 1999b). When comparing species with different diets and ecologies, reliance on stored food per se may not provide an accurate reflection of the memory demands imposed by food caching.

The food-caching black-capped chickadee (*Poecile atricapilla*) occupies an extremely wide geographic range (Harrap & Quinn, 1995). Therefore, comparisons can be made within a single species, in which northern and southern populations might have different degrees of reliance on cached food. Showing that differences in memory exist within different subpopulations of the same species could indicate the evolutionary path for memory and the hippocampus in food-caching animals. In addition, this comparison might provide a tighter control of the adaptive specialization hypothesis by removing some of the confounding between-species variables.

We therefore hypothesized that the selection pressure on caching intensity and cache retrieval efficiency should be greater for birds living in harsher environments, where the food supply is both limited and unpredictable. We predicted that conspecifics wintering in higher latitudes should cache more food and retrieve their caches more efficiently. If the adaptive specialization hypothesis is correct, then the chickadees living in the harsher northern environment should also have more accurate spatial memories for the location of previously hidden food, as well as larger hippocampal volumes containing a greater number of neurons.

To test this hypothesis, we compared food caching, memory-based cache retrieval, and the hippocampal formation of black-capped chickadees from Alaska (Anchorage area) and Colorado (Windsor area). In these locations, the chickadees must endure quite different winter conditions (see Table 1).

Table 1  
*Weather Parameters for the Two Geographical Locations From Which Experimental Black-Capped Chickadees Were Collected*

Parameter	Anchorage, Alaska	Windsor, Colorado
Average temperature (°C)	-5.2	+1.0
Average high temperature (°C)	-2.0	+8.7
Average low temperature (°C)	-9.2	-8.0
Average precipitation (cm)	2.2	1.3
Average snowfall (cm)	26.7	14.5
Average no. of rainy days (per month)	9.3	1.3
Average no. of days below -17 °C (per month)	6.4	1.0
Day length, December 22	5 hr 27 min	9 hr 16 min

*Note.* All weather data are averages for six months (October–March) for multiple years (last 42 years from Anchorage and last 65 years for Windsor; complete data are available from <http://www.weatherbase.com>).

## Method

Fifteen black-capped chickadees (*Poecile atricapilla*) were caught near Anchorage, Alaska (61° 10' N, 150° 1' W) on October 2, 2000, and 12 black-capped chickadees were caught near Windsor, Colorado (40° 35' N, 105° 8' W) on October 18, 2000, by means of mist nets placed near bird feeders. Within a week after capture, the birds were transported (by airplane from Alaska and by car from Colorado) in transport cages to the laboratory at the University of California, Davis and placed individually in wire mesh cages (60 cm long × 42 cm high × 60 cm wide). All birds were maintained on an 8:16-hr light–dark cycle at a constant temperature of 20 °C. Chickadees from Alaska and Colorado were kept in the same room, separated only by a testing chamber. Birds were fed with a mixture of shelled sunflower seeds, crushed peanuts, and mealworms and were given water with vitamins ad libitum.

After the birds had been maintained in captivity for 45 days, we tested their memory performance in a cache-recovery task, followed by three versions of a one-trial associative learning task. For these tests, we used only 8 birds from Alaska and 8 birds from Colorado. The remaining 7 birds from Alaska and 4 birds from Colorado were maintained in identical conditions, except they received no experience in caching and retrieving during the duration of the experiment. This division of birds into two groups allowed us to test whether food caching and recovery experience affects hippocampal size. Before the behavioral tests, all birds were individually acclimated to the experimental room for 2 hr each during two separate sessions. During these sessions, food was provided ad libitum, and the birds were allowed to freely eat and cache. We also placed seeds in caching sites so that birds learned to look for food in these sites. Chickadees from Colorado were significantly larger and heavier than chickadees from Alaska (see Table 2). However, there were no significant differences between the two treatment subgroups used in behavioral experiments in either body size (wing length: Alaska, 67.12 ± 0.54 mm; Colorado, 69.12 ± 1.80 mm;  $n_1 = n_2 = 8$ ),  $t(14) = -2.08$ ,  $p = .06$ , or body mass (Alaska: 11.56 ± 0.54 g, Colorado: 12.28 ± 0.92 g;  $n_1 = n_2 = 8$ ),  $t(14) = -1.91$ ,  $p = .08$ .

## Experimental Room

All birds were tested individually in an experimental room (325 cm long × 218 cm high × 312 cm wide) and observed through a one-way Plexiglas window. The room contained 70 evenly spaced caching sites. There were two trees that contained 20 caching holes each and 30 caching blocks, which were hung from a wire mesh screen attached to the two

Table 2  
*Details of the Sampling Scheme for the Cavalieri Method Used to Estimate Hippocampal Formation and Remaining Telencephalon Volumes and for the Optical Fractionator Method Used to Estimate the Total Number of Hippocampal Formation Neurons*

Parameter	Alaska chickadees (n = 14)	Colorado chickadees (n = 11)
Volume estimation		
Hippocampal formation		
Grid size ( $\mu\text{m}$ )	200	200
Mean no. of points	749.4 $\pm$ 35.6	626.7 $\pm$ 24.8
Telencephalon		
Grid size ( $\mu\text{m}$ )	1,142.86	1,142.86
Mean no. of points	315.4 $\pm$ 11.4	393.5 $\pm$ 11.9
Neuron counts		
Dissector height ( $\mu\text{m}$ )	6	6
Step area ( $\mu\text{m}^2$ )	62,500	62,500
Frame area ( $\mu\text{m}^2$ )	900	900
Section sampling fraction	0.08	0.08
Area sampling fraction	0.01	0.01
Thickness sampling fraction	0.530 $\pm$ 0.003	0.540 $\pm$ 0.010

opposite walls of the room (15 blocks on each wall). Each caching block consisted of a wooden rectangle (8.0 cm long  $\times$  14.0 cm high  $\times$  3.5 cm wide) with a hole in the center. Holes in the trees and in the wooden blocks were 0.5 cm in diameter and 0.5 cm deep. Each hole could be covered by a knot at the end of a short string attached above the hole to prevent the birds from seeing its contents. A wooden perch was located below each hole. The birds readily cached food in all caching sites and inspected them for food.

The experimenter manipulated the lights and opened the flaps connecting the home cages with the room so that the birds could fly in and out of the experimental room by flying from the darkness to the light. Thus, at the start of a trial, the lights were turned off in the home cage and turned on in the experimental room; at the end of the trial, the lights were reversed, and the bird would fly into the home cage. By using this procedure, we avoided handling-induced stress in the birds.

### Cache-Recovery Task

The cache-recovery task consisted of two phases separated by a retention interval of 5 hr. Each bird was deprived of food for 1 hr before night roost and for 1 hr before Phase 1, in which birds were allowed to eat and to cache food individually for 20 min in the experimental room, with 70 available caching sites. During Phase 1, an unlimited amount of sunflower seeds was provided in a bowl. After Phase 1, birds were returned to their home cages, where food (crushed peanuts and sunflower seeds) was available ad libitum. They were then deprived of food for 2 hr before Phase 2. During Phase 2, birds were allowed in the experimental room for 10 min, and the only food available was that which they had cached during Phase 1. During Phase 2, all 70 caching holes were covered with a string knot so that the contents of caching sites were not visible. To retrieve a cache or to inspect the hole, a bird had to pull the string away from the hole. Before Phase 2, we placed all caches back in their original caching sites.

During Phase 1 (storage), we recorded the number of seeds cached and where they were located. During Phase 2 (recovery), we recorded the number of caches each bird recovered and the number of looks needed to find each seed. A bird was recorded as having made a look when it pulled at the string covering one of the potential cache sites. Each bird was tested individually for two trials from November 27, 2000 until December 10,

2000. For statistical analyses, we used individual birds' means from both trials.

### One-Trial Associative Learning Task

After the cache-recovery trials were completed, we also tested each bird individually in a one-trial associative learning task. In this task, birds were trained to search, in Phase 2 of a trial, for food in sites where they had previously found food during Phase 1 (Clayton, 1995a; Clayton & Krebs, 1994; Pravosudov & Clayton, 2001). One problem with the cache-recovery task is that if birds differ in the number of caches they make, it is more difficult to directly compare the birds' accuracy of cache recovery. In the one-trial associative learning task, all birds have exactly the same experience because the experimenter effectively hides the caches and therefore controls for the number of items that need to be remembered. This is particularly pertinent for the comparison between Alaska and Colorado chickadees, given that the two populations are likely to differ in the number of items they cache in the wild.

We used three versions of the one-trial associative learning task. In all versions, only one site was baited with food. In Version 1 (December 15–18, 2000), birds had 70 sites available to them, exactly as in the cache-recovery task, including the same number of sites in trees and wooden blocks. In Version 2 (December 19–22, 2000), we limited the available sites to the 15 wooden blocks. In both task versions, the birds had to rely on memory to accurately return to the site that contained food. To examine the effect of motivation on performance, we also tested birds in a third version (December 23–24, 2000). We used 15 cache sites as in Version 2, but this time the baited site was color-marked with a unique green pattern so the birds did not have to rely on spatial memory to remember which feeder contained the hidden food in Phase 2. If birds differed in their motivation to search for food, then they should have differed in performance on all three versions of the task. With 15 sites versus 70 sites, there should have been less searching for food because there were fewer sites. With the color-marked feeding site, birds should not have had to search for food and instead should have searched only for the marked site. If the two treatment groups differed in memory, but not motivation, then they should have differed in performance in Versions 1 and 2, but not in Version 3.

Each trial consisted of two phases separated by a retention interval of 60 min. In Phase 1, each bird had to locate a visible piece of peanut (which all birds did immediately without looking anywhere else). Each bird was allowed to eat a small part of the peanut for 30 s, after which the lights in the experimental room were turned off and the bird was returned to its home cage. All birds were deprived of food for 1 hr before night roost, for 1 hr before Phase 1, and between Phase 1 and Phase 2. In Phase 2, all caching sites were covered with a knot at the end of the string. If the bird remembered precisely where it had encountered the food, it should go directly to the site from which it had partially consumed the peanut in Phase 1 (Clayton & Krebs, 1994; Healy & Krebs, 1992). The site containing peanut in Phase 1 also contained pieces of peanut covered by a string knot, so the birds were rewarded for a successful search. We recorded the number of sites inspected during Phase 2 for each bird.

### Brain Analyses

All birds were anesthetized (0.03 ml per bird of 50 mg/ml Nembutal-sodium solution) and perfused transcardially with 100 ml of phosphate buffer (0.1 M  $\text{PO}_4$ ) followed by 100 ml of 4% (wt/vol) paraformaldehyde in phosphate buffer. All birds were sexed after perfusion. Birds were decapitated and their brain (within the skull) was placed in 4% paraformaldehyde for 1 week. We then removed the brains from the skull and postfixed them in 4% paraformaldehyde for another week. All brains were cryoprotected in a 30% (wt/vol) sucrose solution, frozen, and kept at  $-20^\circ\text{C}$  until processing. We cut coronal sections at 40  $\mu\text{m}$  on a sliding,

freezing microtome and collected every fourth section in phosphate-buffered saline (0.1 M, pH 7.4). Every fourth section was mounted onto gelatin-coated slides, Nissl-stained with thionin, and cover-slipped with Permount. For the brain analyses, we used both birds that had participated in behavioral experiments and birds that had not participated in behavioral experiments. Because of some technical problems, however, only 25 chickadees were used for measurements of volume and neuron number: 14 from Alaska and 11 from Colorado.

We used StereoInvestigator software (Version 3.15a, Microbrightfield, Colchester, VT) for all stereological measurements. We used the Cavalieri principle to measure the volume of the hippocampal formation and the volume of the telencephalon minus the hippocampal formation on the Nissl-stained sections (Gundersen & Jensen, 1987; West & Gundersen, 1990). We determined the boundaries of the hippocampal formation as described in Krebs et al. (1989). To evaluate the precision of our sampling methods, we calculated coefficients of error for volume and neuron count measurements. The relative variance of individual estimates was calculated by using the quadratic approximation (Gundersen & Jensen, 1987) and taking into account the Nugget effect (West, Ostergaard, Andreassen, & Finsen, 1996). We used a total of 11–15 sections per bird to measure the hippocampal volume (480  $\mu\text{m}$  apart, or 1 in 12 sections). In accordance with the Cavalieri principle (Gundersen & Jensen, 1987; West & Gundersen, 1990), we chose the first section randomly from the first 4 sections containing the hippocampus. Pilot studies showed that this sampling scheme was most efficient and provided very low variance of individual estimates (see Table 3), and that increasing the number of sections measured did not increase estimation accuracy. To measure the volume of the telencephalon, we used a total of 11–15 sections (640  $\mu\text{m}$  apart, or 1 in 16 sections), with the first section chosen randomly from the first 6 sections. Sections were coded before the analyses, and the code was broken after all measurements were completed. Details of the sampling scheme for the Cavalieri method are presented in Table 2.

We calculated the total number of neurons in the hippocampal formation by using the optical fractionator method on the Nissl-stained sections (West et al., 1991). The optical fractionator method allows one to estimate the absolute number of neurons independently from the volume of the structure (West et al., 1991). To estimate the total number of neurons in the hippocampal formation, we used the same sections used for the volumetric measurements. To count neurons, we used a  $\times 100$  Neofluar oil objective (N.A. 1.30) on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator. We estimated the number of neurons in the right half of the hippocampus and then doubled the number to get the total number of neurons in the hippocampal formation, as there were no significant size differences between the right and left sides: sign test,  $z = 0.8$ ,  $p = .42$ ,  $n = 25$ . Details of the sampling scheme for the optical fractionator method are presented in Table 2.

We used *t* tests and analyses of covariance (ANCOVAs) for all behavioral data, as well as to compare the volume of the hippocampal formation and the remainder of the telencephalon. To compare hippocampal volume with the volume of the remainder of the telencephalon, we performed an ANCOVA on the log-transformed (natural logarithm) volume measurements, with nonhippocampal telencephalon volume as a covariate. All data (raw or log-transformed) met the assumptions for parametric statistical procedures.

## Results

### Cache-Recovery Task

During Phase 1 of the cache-recovery task, both Alaska and Colorado chickadees consumed similar amounts of food,  $t(14) = 0.96$ ,  $p = .35$ ,  $n_1 = n_2 = 8$  (see Figure 1), but Alaska birds cached significantly more seeds than Colorado birds,  $t(14) = 2.47$ ,  $p < .03$ ,  $n_1 = n_2 = 8$  (see Figure 1).

Both Alaska and Colorado chickadees located their caches significantly better (inspected fewer number of sites in order to locate their caches) than would be expected from random search: paired *t* test, Alaska,  $t(7) = -2.52$ ,  $p < .05$ ,  $n = 8$ ; Colorado,  $t(7) = -4.39$ ,  $p < .01$ ,  $n = 8$ . Independent of the number of caches made by each bird, Alaska chickadees inspected significantly fewer sites in order to locate those containing caches than did Colorado birds during Phase 2 of the cache-recovery task: ANCOVA,  $F(1, 13) = 23.83$ ,  $p < .01$ ; number of caches as a covariate,  $p = .51$  (see Figure 2). In addition, we also compared the efficiency of finding hidden caches (i.e., number of looks needed to find a cache), corrected for the expected probability of finding different numbers of caches made by individual birds assuming random search (efficiency divided by expected probability of finding caches using random search). We obtained a very similar pattern of results: Alaska chickadees inspected fewer sites in order to find their cached seeds compared with Colorado chickadees,  $t(14) = -4.39$ ,  $p < .001$ ,  $n_1 = n_2 = 8$ . In both groups of chickadees, there was no significant relationship between the number of caches made by different individuals and either cache-retrieval efficiency or the mean number of looks needed to find a site containing a cache: regression, Alaska,  $F(1, 6) = 0.48$ ,  $p = .51$ ; Colorado,  $F(1, 6) = 0.21$ ,  $p = .66$ ;  $n_1 = n_2 = 8$ .

Table 3

*Wing Length, Body Mass, Volume of the Hippocampal Formation and the Remainder of the Telencephalon, and Total Number of Neurons in the Hippocampus*

Parameter	Alaska chickadees	CE (range)	Colorado chickadees	CE (range)	<i>t</i> test results
Wing length (mm)	66.67 $\pm$ 0.75		69.33 $\pm$ 0.50		$t(25) = -3.34$ , $p < .001$
Body mass (g)	11.38 $\pm$ 0.18		12.28 $\pm$ 0.25		$t(23) = -3.13$ , $p < .01$
Hippocampal formation volume (mm <sup>3</sup> )	14.39 $\pm$ 0.68	0.02 (0.01–0.03)	12.18 $\pm$ 0.43	0.02 (0.01–0.03)	$t(23) = 2.56$ , $p = .02$
Remainder of telencephalon volume (mm <sup>3</sup> )	262.84 $\pm$ 9.25	0.02 (0.01–0.03)	328.67 $\pm$ 9.99	0.02 (0.01–0.03)	$t(23) = -4.81$ , $p < .001$
Total no. of neurons	1,389,052 $\pm$ 48,901	0.06 (0.05–0.07)	952,534 $\pm$ 42,079	0.06 (0.05–0.08)	$t(23) = 6.55$ , $p < .001$

*Note.* For Alaska chickadees,  $n = 15$  for wing length and body mass, and  $n = 14$  for all other measures. For Colorado chickadees,  $n = 12$  for wing length and body mass, and  $n = 11$  for all other measures. CE represents the average (per group) of the relative variance of individual estimates of volumetric brain measures and the total number of neurons.

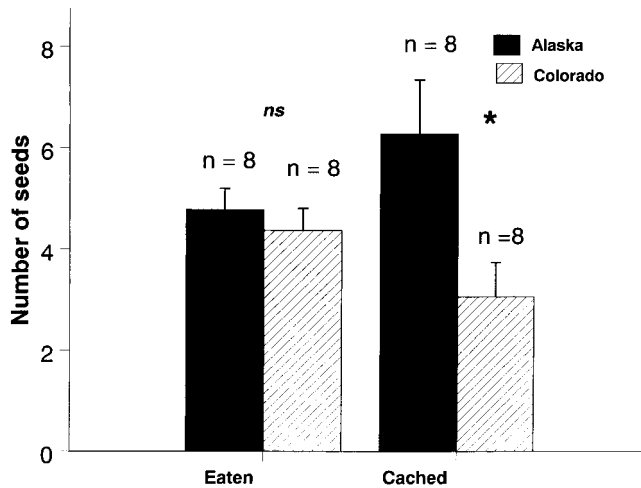


Figure 1. Mean ( $\pm$  SEM) number of sunflower seeds eaten and cached during Phase 1 of the cache-recovery task. Asterisk indicates a significant difference between groups ( $p < .05$ ); ns = nonsignificant.

### One-Trial Associative Learning Task

When all 70 sites were available, as in the cache-recovery task, both Alaska and Colorado chickadees performed significantly better (inspected significantly fewer sites) than would be expected from random search: paired  $t$  test, Alaska,  $t(7) = -23.8$ ,  $p < .001$ ,  $n = 8$ ; Colorado,  $t(6) = -21.61$ ,  $p < .001$ ,  $n = 7$ . However, Alaska chickadees inspected fewer sites in order to find the peanut pieces in Phase 2 compared with Colorado chickadees,  $t(13) = -2.82$ ,  $p < .02$ ,  $n_1 = 8$ ,  $n_2 = 7$  (see Figure 3). When we reduced the number of available sites in which birds could search for food to 15, Alaska chickadees also showed better performance on the one-trial associative learning task compared with Colorado chickadees,  $t(11) = -2.66$ ,  $p < .03$ ;  $n_1 = 7$ ,  $n_2 = 6$  (see Figure 3). However, both groups performed significantly better than would

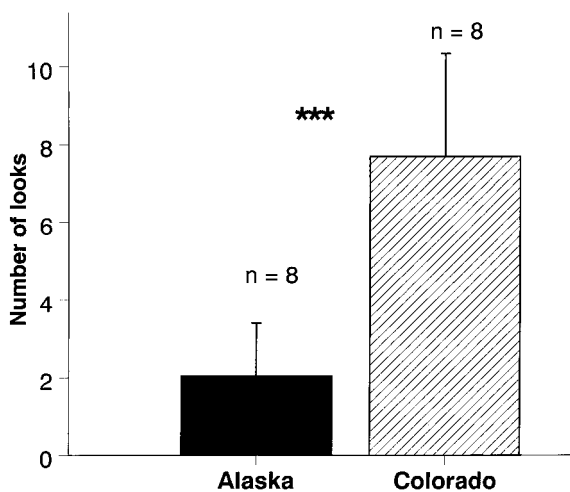


Figure 2. Mean ( $\pm$  SEM) number of sites inspected in Phase 2 of the cache-recovery task per one successful cache retrieval. Asterisks indicate a significant difference between groups ( $p < .001$ ).

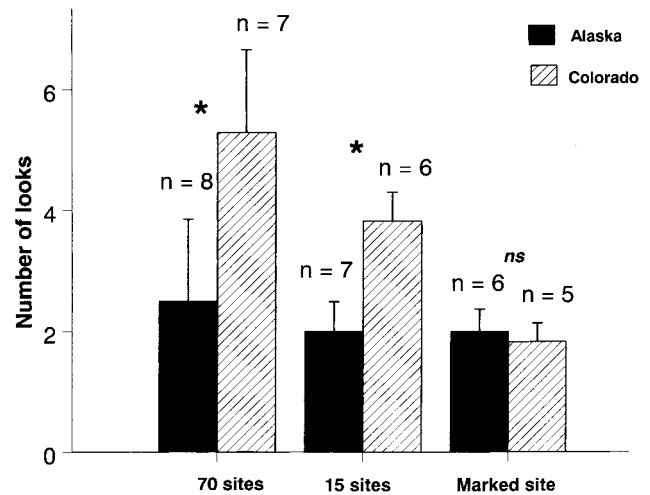


Figure 3. Mean ( $\pm$  SEM) number of sites inspected during Phase 2 of a one-trial associative learning task before finding a site in which food was previously found in Phase 1. Data are presented for three versions of the task: Version 1 (all 70 sites available), Version 2 (only 15 sites available), and Version 3 (15 sites available, but the site containing peanut pieces was color-marked). Asterisks indicate a significant difference between groups ( $p < .01$ ); ns = nonsignificant.

be expected from random search: paired  $t$  test, Alaska,  $t(6) = -11.27$ ,  $p < .001$ ,  $n = 7$ ; Colorado,  $t(5) = -7.68$ ,  $p < .001$ ,  $n = 6$ . However, when the feeder containing peanut was marked with a unique color pattern that made the search easier (because birds could rely on spatial, color, and pattern cues to discriminate one feeder from the rest), the differences between Alaska and Colorado chickadees disappeared,  $t(9) = 0.65$ ,  $p = .53$ ,  $n_1 = 6$ ,  $n_2 = 5$  (see Figure 3).

### Volumetric Data

Alaska chickadees had significantly smaller telencephalon volumes (having had the volume of the hippocampal formation removed) compared with Colorado chickadees (see Table 2). These differences were not surprising, as Alaska chickadees were also significantly smaller and weighed significantly less than Colorado chickadees (see Table 2). Wing length and body mass were highly positively correlated across all birds: regression,  $F(1, 25) = 37.60$ ,  $p < .001$ , as were telencephalon volume and either wing length: regression,  $F(1, 23) = 8.30$ ,  $p < .01$ , or body mass: regression,  $F(1, 23) = 7.88$ ,  $p < .01$ . Despite having smaller body size and smaller telencephalon, the absolute volume of the hippocampal formation was significantly larger in Alaska chickadees compared with Colorado chickadees (see Table 2). Thus, the volume of the hippocampal formation relative to the remainder of the telencephalon was also significantly larger in Alaska chickadees: ANCOVA,  $F(1, 22) = 47.90$ ,  $p < .001$  (see Figure 4). The volume of the hippocampal formation covaried positively with the volume of the remainder of the telencephalon within both groups: ANCOVA,  $F(1, 22) = 34.10$ ,  $p < .001$  (see Figure 4).

Alaska chickadees also had significantly more neurons in their hippocampal formation than did Colorado chickadees (see Table 2). When corrected for the volume of the remainder of the telen-

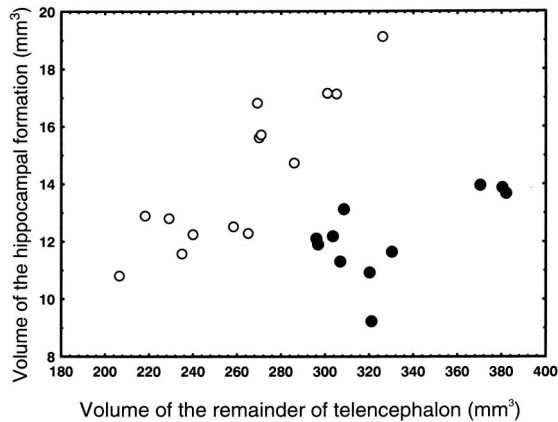


Figure 4. The relationship between the hippocampal formation volume and the remainder of the telencephalon volume in Alaska (open circles) and Colorado (filled circles) black-capped chickadees.

cephalon, the difference between Alaska and Colorado birds in the total number of hippocampal neurons became even more significant: ANCOVA, group,  $F(1, 22) = 64.50, p < .001$ ; telencephalon minus hippocampal formation,  $F(1, 22) = 11.70, p < .001$ .

There were no significant differences between males and females in telencephalon minus hippocampal formation volume: ANOVA with treatment and sex as independent variables, sex,  $F(1, 21) = 1.00, p = .33$ ; relative hippocampal volume: ANOVA with treatment and sex as independent variables and remainder of the telencephalon as a covariate, sex,  $F(1, 20) < 1.00, p = .85$ ; or the total number of hippocampal neurons: ANOVA with treatment and sex as independent variables and telencephalon minus hippocampal formation volume as a covariate, sex,  $F(1, 20) < 1.00, p = .39$ .

For both Alaska and Colorado chickadees, we compared the hippocampal volumes of those birds that had the experience of caching and retrieving during the experiment with other birds that did not have such experience yet were caught at the same time and had been maintained in the laboratory for the same length of time. We found that caching and retrieval experience had no significant effect on the relative volume of the hippocampal formation: ANCOVA,  $F(1, 20) = 1.20, p = .29$ , or on the total number of hippocampal neurons,  $F(1, 20) < 1.00, p = .98$ . An interaction between caching experience and group also was not significant for either hippocampal formation,  $F(1, 20) < 0.10, p = .75$ , or total neuron number,  $F(1, 20) < 1.00, p = .55$ , whereas the group effect remained highly significant,  $F(1, 20) > 41.00, p < .001$ . The variance of our volume and neuron number estimates remained very low for all measurements (see Table 2), which supports the reliability of our methods.

### Discussion

There are five main findings of our study. First, black-capped chickadees from Alaska cached significantly more food than did black-capped chickadees from Colorado. Second, chickadees from Alaska needed fewer looks to find hidden food compared with Colorado birds on both the cache-recovery task and the first two one-trial associative learning tasks. Third, there were no signifi-

cant differences between the two groups in the third version of the one-trial associative learning task, in which the feeder containing peanut pieces was marked with a unique color pattern. Fourth, Alaska chickadees had significantly larger hippocampal volumes containing significantly more neurons than did Colorado chickadees. Fifth, experience of caching and retrieving food in the laboratory over the course of the experiment had no significant effect on the hippocampal volume or on the total number of hippocampal neurons in Alaska and Colorado chickadees.

The fact that Alaska chickadees cached significantly more food than Colorado chickadees under laboratory conditions supports the prediction that birds living in higher latitudes should cache more food than birds living in lower latitudes because the benefits of cached food are greater for those that live in harsher and more unpredictable environments (Brodin, Lahti, Lens, & Suhonen, 1996; Pravosudov & Grubb 1997a). Brodin et al. (1996) failed to find a difference in caching intensity between northern and southern populations of free-ranging willow tits under natural conditions, however, which is contradictory to the results that we obtained. It is possible that differences in natural conditions in that study (yearly variation in food supply, weather, etc.) confounded the results such that the difference in caching rates between northern and southern populations was minimized. Our populations were farther apart geographically than those in the Brodin et al. (1996) comparison, and we compared them under identical conditions in a controlled laboratory environment.

The results of our experiment also support the prediction that Alaska chickadees would be more accurate at cache retrieval than Colorado chickadees. Indeed, the Alaska chickadees needed significantly fewer visits to find a cache. During the cache-recovery trial, however, Alaska chickadees cached more food than did Colorado chickadees. Consequently, they might have had higher cache-retrieval efficiency even if they searched at random, because the probability of finding a cache by random search increases with the number of items cached. However, the cache-retrieval accuracy of Alaska chickadees was significantly higher even when we corrected for the expected probability of finding a different number of caches at random. Consistent with the results from the cache-recovery test, the Alaska chickadees also performed more accurately on spatial memory tests than did chickadees from Colorado.

However, there are a number of reasons that might explain why chickadees from Alaska and Colorado showed differences in performance in the memory tasks that we used. The Alaska birds might have been more motivated to search for food, or they might possess a more accurate memory of the locations of their caches, given their greater reliance on caches in the wild. Enhanced performance on memory tasks could also have occurred if Alaska birds paid more attention to the cache sites during caching and therefore encoded the information more accurately, or because they were more accurate at recalling the sites in which they cached food. Our results do not distinguish between differences in attention at encoding and differences in memory recall.

It is possible that Alaska and Colorado chickadees differed in their motivation to recover their hidden food caches. Although birds from Alaska cached significantly more food compared with Colorado birds, there were no significant differences in the amount of food consumed by the birds in both groups, which suggests that they had similar hunger levels. Birds in both groups were suffi-

ciently motivated to search for hidden food, and when Alaska and Colorado chickadees were tested for motivation levels in the nonspatial version of the one-trial associative learning task (in which a baited site was uniquely color marked), there was no difference between the groups. Although this result suggests that the two groups were equally motivated to search for hidden food, it is possible that the test was not sensitive enough to detect possible differences in motivation.

Although environmental conditions of chickadees from Alaska and Colorado may differ in a number of ways, the most drastic differences appear to be in harshness and unpredictability of foraging during the winter. Perhaps the most critical factor affecting selection pressure on food caching and spatial memory in these birds is day length. Alaska chickadees must obtain enough food to survive the night during half the time available to Colorado chickadees. Such a difference in time available to forage might, by itself, select for more caching and more accurate cache retrieval, but it is additionally compounded by differences in temperature and snowfall. Dynamic models also predict that reduction in food availability should result in increased caching intensity and that more accurate cache retrieval should increase survival probability in food-caching birds (Pravosudov & Lucas, 2001). To separate the immediate, short-term effects (as mediated, for example, by differences in hunger that arise as a consequence of differences in the number of hours available for eating) from evolutionary, long-term effects of day length on food caching and memory, it is crucial to compare food-caching behavior and memory performance in birds maintained on the same photoperiod.

In the milder Colorado conditions, birds might not need to constantly maintain superior memory but instead might respond to short-term deterioration in local weather by temporarily improving their cache-retrieval accuracy, possibly mediated by elevated baseline levels of corticosterone (Pravosudov & Clayton, 2001; Pravosudov, Kitaysky, Wingfield, & Clayton, 2001). Birds that constantly experience energetically demanding conditions, like Alaskan black-capped chickadees, appear to have developed mechanisms that allow for permanent differences in spatial memory, such as an enlarged hippocampal formation with more neurons. For example, Hampton and Sherry (1992) and Hampton et al. (1995) reported that parid species living in milder climates cache less food and have smaller hippocampi compared with species living in harsher environments. Our results with black-capped chickadees parallel these findings and suggest that harsh environmental conditions provide more selection pressure on food caching and memory for cache retrieval.

Although our data do not allow us to establish whether the behavioral and brain differences between Alaska and Colorado birds have a genetic basis, it is clear that the differences between the two groups are not simply caused by the temporary differences in the local environment during the winter. The differences between Alaska and Colorado chickadees in caching, memory, and hippocampal anatomy persist even after more than a month in captivity under identical conditions. Our results also show that caching and retrieval experience in the lab had no effect on volume and neuron number of the hippocampal formation of black-capped chickadees. This is consistent with the findings of Cristol (1996), who also reported that a 1-month absence of caching experience had no effect on the hippocampal volume in adult willow tits.

Regardless of the origin of these population differences in caching behavior, the enhanced caching intensity, cache-recovery efficiency, and larger hippocampal formation of Alaska chickadees are important adaptations to life in hostile environments, ones that may have helped individuals of this species to extend their range far north.

## References

- Balda, R. P., & Kamil, A. C. (1989). A comparative study of cache recovery by three corvid species. *Animal Behaviour*, *26*, 486–495.
- Balda, R. P., & Kamil, A. C. (1992). Long-term spatial memory in Clark's nutcracker, *Nucifraga columbiana*. *Animal Behaviour*, *44*, 761–769.
- Basil, J. A., Kamil, A. C., Balda, R. P., & Fite, K. V. (1996). Differences in hippocampal volume among food storing corvids. *Brain, Behavior and Evolution*, *47*, 156–164.
- Biegler, R., McGregor, A., Krebs, J. R., & Healy, S. D. (2001). A larger hippocampus is associated with longer-lasting memory. *Proceedings of the National Academy of Sciences, USA*, *98*, 6941–6944.
- Bolhuis, J. J., & Macphail, E. M. (2001). A critique of the neuroecology of learning and memory. *Trends in Cognitive Sciences*, *5*, 426–433.
- Brodin, A. (1994). *Time aspects on food hoarding in the willow tit*. Unpublished doctoral dissertation, Stockholm University, Stockholm, Sweden.
- Brodin, A., Lahti, K., Lens, L., & Suhonen, J. (1996). A northern population of willow tits *Parus montanus* did not store more food than southern ones. *Ornis Fennica*, *73*, 114–118.
- Clayton, N. S. (1995). Development of memory and the hippocampus: Comparison of food storing and nonstoring birds on a one-trial associative memory task. *Journal of Neuroscience*, *15*, 2796–2807.
- Clayton, N. S., & Dickinson, A. (1998, September 17). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*, 272–278.
- Clayton, N. S., & Dickinson, A. (1999a). Memory for the contents of caches by scrub jays. *Journal of Experimental Psychology: Animal Behavior Processes*, *25*, 82–91.
- Clayton, N. S., & Dickinson, A. (1999b). Scrub jays (*Aphelocoma coerulescens*) remember the relative time of caching as well as the location and content of their caches. *Journal of Comparative Psychology*, *113*, 403–416.
- Clayton, N. S., & Krebs, J. R. (1994). One-trial associative memory: Comparison of food-storing and nonstoring species of birds. *Animal Learning and Behavior*, *22*, 366–372.
- Cristol, D. A. (1996). Food storing does not affect hippocampal volume in experienced adult willow tits. *Behavioural Brain Research*, *81*, 233–236.
- Gundersen, H. J. G., & Jensen, E. B. (1987). The efficiency of systematic sampling in stereology and its predictions. *Journal of Microscopy*, *147*, 229–263.
- Haftorn, S. (1956). Contribution to the food biology of tits, especially about storing of surplus food: Part IV. A comparative analysis of *Parus atricapillus* L., *P. cristatus* L., and *P. ater* L. *Det Kongelige Norske Videnskabers Selskabs Skrifter*, *4*, 1–54.
- Hampton, R. R., & Sherry, D. F. (1992). Food storing by Mexican chickadees and bridled titmice. *Auk*, *109*, 665–666.
- Hampton, R. R., Sherry, D. F., Shettleworth, S. J., Khurgel, M., & Ivy, G. (1995). Hippocampal volume and food-storing behavior are related in parids. *Brain, Behavior and Evolution*, *45*, 54–61.
- Harrap, S., & Quinn, D. (1995). *Chickadees, tits, nuthatches and tree-creepers*. Princeton, NJ: Princeton University Press.
- Healy, S. D., & Krebs, J. R. (1992). Food-storing and hippocampus in corvids: Amount and volume are correlated. *Proceedings: Biological Sciences*, *248*, 241–245.
- Healy, S. D., & Krebs, J. R. (1996). Food-storing and the hippocampus in *Paridae*. *Brain, Behavior and Evolution*, *47*, 195–199.

- Hurly, T. A. (1992). Energetic reserves of marsh tits (*Parus palustris*): Food and fat storage in response to variable food supply. *Behavioral Ecology*, 3, 181–188.
- Kamil, A. C., Balda, R. P., & Olson, D. J. (1994). Performance of four seed-caching corvid species in the radial-arm maze analog. *Journal of Comparative Psychology*, 108, 385–393.
- Krebs, J. R. (1990). Food-storing birds: Adaptive specialization in brain and behavior. *Philosophical Transactions: Biological Sciences*, 329, 153–160.
- Krebs, J. R., Clayton, N. S., Healy, S. D., Cristol, D. A., Patel, S. N., & Jolliffe, A. R. (1996). The ecology of the avian brain: Food-storing memory and the hippocampus. *Ibis*, 138, 34–46.
- Krebs, J. R., Sherry, D. F., Healy, S. D., Perry, V. H., & Vaccarino, A. L. (1989). Hippocampal specialization of food-storing birds. *Proceedings of the National Academy of Sciences, USA*, 86, 1388–1392.
- Macphail, E. M., & Bolhuis, J. J. (2001). The evolution of intelligence: Adaptive specializations versus general process. *Biological Reviews*, 76, 341–364.
- McNamara, J. M., Houston, A. I., & Krebs, J. R. (1990). Why hoard? The economics of food caching in tits, *Parus* spp. *Behavioral Ecology*, 1, 12–23.
- Olson, D. J., Kamil, A. C., Balda, R. P., & Nims, P. J. (1995). Performance of four seed-caching corvid species in operant tests of non-spatial and spatial memory. *Journal of Comparative Psychology*, 109, 173–181.
- Pravosudov, V. V. (1985). Search for and storage of food by *Parus cinctus lapponicus* and *P. montanus borealis* (Paridae). *Zoologicheskii Zhurnal*, 64, 1036–1043.
- Pravosudov, V. V., & Clayton, N. S. (2001). Effects of demanding foraging conditions on cache retrieval accuracy in food-caching mountain chickadees (*Poecile gambeli*). *Proceedings: Biological Sciences*, 268, 363–368.
- Pravosudov, V. V., & Grubb, T. C., Jr. (1997a). Energy management in passerine birds during the non-breeding season: A review. *Current Ornithology*, 14, 189–234.
- Pravosudov, V. V., & Grubb, T. C., Jr. (1997b). Management of fat reserves and food caches in tufted titmice (*Parus bicolor*) in relation to unpredictable food. *Behavioral Ecology*, 8, 332–339.
- Pravosudov, V. V., Kitaysky, A. S., Wingfield, J. C., & Clayton, N. S. (2001). Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *General and Comparative Endocrinology*, 123, 324–331.
- Pravosudov, V. V., & Lucas, J. R. (2001). A dynamic model of short-term energy management in small food-caching and non-caching birds. *Behavioral Ecology*, 8, 207–218.
- Sherry, D. F., & Vaccarino, A. L. (1989). Hippocampus and memory for food caches in black-capped chickadees. *Behavioral Neuroscience*, 103, 308–318.
- Sherry, D. F., Vaccarino, A. L., Buckenham, K., & Hertz, R. S. (1989). The hippocampal complex of food-storing birds. *Brain, Behavior and Evolution*, 34, 308–317.
- Shettleworth, S. J. (1995). Memory in food-storing birds: From the field to the Skinner box. In E. Alleva, A. Fasolo, H. P. Lipp, L. Nadel, & L. Ricceri (Eds.), *Behavioral brain research in naturalistic and semi-naturalistic settings* (pp. 158–179). The Hague, the Netherlands: Kluwer.
- Shettleworth, S. J., & Hampton, R. R. (1998). Adaptive specializations of spatial cognition in food storing birds? Approaches to testing a comparative hypothesis. In I. Pepperberg, R. Balda, & A. Kamil (Eds.), *Animal cognition in nature* (pp. 65–98). San Diego, CA: Academic Press.
- Vander Wall, S. B. (1990). *Food hoarding in animals*. Chicago: University of Chicago Press.
- Volman, S. F., Grubb, T. C., Jr., & Schuett, K. C. (1997). Relative hippocampal volume in relation to food-storing behavior in four species of woodpeckers. *Brain, Behavior and Evolution*, 49, 110–120.
- West, M. J., & Gundersen, H. J. G. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *Journal of Comparative Neurology*, 296, 1–22.
- West, M. J., Ostergaard, K., Andreassen, O. A., & Finsen, B. (1996). Estimation of the number of somatostatin neurons in the striatum: An in situ hybridization study using the optical fractionator method. *Journal of Comparative Neurology*, 370, 11–22.
- West, M. J., Slomianka, L., & Gundersen, H. J. G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anatomical Record*, 231, 482–497.

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