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No latitudinal differences in adrenocortical stress response in wintering black-capped chickadees (*Poecile atricapilla*)

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Abstract

Birds respond to deterioration in environmental conditions by elevating their corticosterone levels, which can enhance their survival. It is less clear if animals constantly living in energetically challenging environment show similar increases in adrenocortical function. Previous work has demonstrated that under controlled conditions black-capped chickadees (*Poecile atricapilla*) from northern latitudes cache more food and perform better on spatial memory tasks than their southern conspecifics. As elevated levels of corticosterone have been shown previously to correlate with spatial memory performance in chickadees, this study aimed to investigate whether black-capped chickadees from northern latitudes have elevated baseline levels of corticosterone and/or a stronger adrenocortical stress response than their southern conspecifics, irrespective of their immediate environment. We found no differences between Alaskan and Colorado chickadees maintained under identical conditions for 3 months in either baseline levels of corticosterone or maximum levels of corticosterone achieved during the stress response. Baseline corticosterone levels were negatively correlated with relative body mass across both groups of birds. Our results suggest that the population differences in food caching behavior and spatial memory were not related to differences in corticosterone levels. We conclude that many reported population differences in baseline levels and in strength of adrenocortical stress response may often reflect differences in local environmental conditions rather than population-specific physiological traits.

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Keywords: Corticosterone; Stress response; Black-capped chickadee; *Poecile atricapilla*; Latitudinal differences; Spatial memory

1. Introduction

It is well known that glucocorticoid hormones may mediate various aspects of animal behavior (e.g. Wingfield et al., 1995, 1997, 1998; Silverin, 1998). Much work has been focused specifically on how changes in environmental conditions affect

levels of glucocorticoids and how elevated levels of glucocorticoids affect animal behavior (e.g. Wingfield et al., 1998; Silverin, 1998). It has been demonstrated, for example, that sudden deteriorations in the environment (e.g. snow storm, air temperature drop, etc.) may result in a significant elevation of corticosterone secretion and behavioral changes directed toward immediate survival in birds (Wingfield et al., 1998). Most of the work has concentrated specifically on the adrenocortical stress response in breeding birds and factors affecting such a response (Wingfield et al., 1995, 1997,

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1998; Silverin, 1998). Less attention has been paid to the variation in levels of corticosterone during winter although there is an indication that wintering resident food caching birds may maintain slightly elevated baseline levels of corticosterone for extended periods of time (Silverin, 1998). Whereas it appears that winter elevation in baseline corticosterone levels is rather uncommon among animals (Romero, 2002), most of the bird species reported in Romero's (2002) extensive review of seasonal variation in glucocorticoids are migratory and there are very limited data on permanent resident birds. Resident birds do not leave breeding areas during the winter and thus must survive conditions characterized by limited and unpredictable food supplies, short day length and low ambient temperatures. Limited and unpredictable food supplies, in particular, appear to be responsible for winter elevations in baseline levels of corticosterone (Pravosudov et al., 2001). Elevations in baseline levels of corticosterone that are triggered by environmental conditions may be highly adaptive by mediating changes in energy management. In resident food-caching birds, for example, individuals that receive limited and unpredictable food elevated corticosterone levels and showed increased efficiency at cache retrieval and enhanced performance on spatial memory tasks when compared to those maintained on ad libitum food (Pravosudov and Clayton, 2001; Pravosudov et al., 2001). There are notable species-specific differences in baseline levels of plasma corticosterone, which presumably evolved to meet a specific niche for each species (Wingfield et al., 1998). Within a species, animals from different populations also show different adrenocortical activity (Wingfield et al., 1998). However, it is not always clear whether differences in the adrenocortical activity between populations reflect current environmental conditions or whether animals constantly living in different environments develop permanent changes in their physiology. In the latter case we would expect the adrenocortical activity of individuals from different populations to differ even if immediate environmental conditions would be similar. So, given that increased baseline levels of corticosterone might enhance memory performance in food caching birds (Pravosudov and Clayton, 2001; Pravosudov et al., 2001), we can ask whether populations with higher selection pressure on memory would evolve permanently elevated

baseline levels of corticosterone. Recent studies suggested that to fully understand whether adrenocortical function has evolved to meet specific niche requirements we need to measure the levels of corticosterone binding proteins and target tissue receptor numbers in addition to measuring total levels of corticosterone (Breuner and Orchinik, 2002). However, measuring total plasma corticosterone levels remains an important first step.

One way to investigate if environmental conditions can result in permanent changes in adrenocortical activity would be to compare different remote populations of the same resident species, which constantly occur under different environmental conditions. For example, the black-capped chickadee (*Poecile atricapilla*) occupies an extremely broad range extending from New Mexico to Alaska (Harrap and Quinn, 1995), and it is a resident food caching species that does not show regular migration (Harrap and Quinn, 1995). Birds living in Alaska experience much more energetically stressful conditions than birds living farther south because of long winters, short days, colder air temperature and more snow cover (Pravosudov and Clayton, 2002). Furthermore, when housed under identical laboratory conditions, black-capped chickadees from Alaska cached significantly more food, retrieved their caches more efficiently and performed significantly better on spatial memory tests than chickadees from the more southerly population in Colorado (Pravosudov and Clayton, 2002). The Alaskan chickadees were found to have significantly larger hippocampal volumes with more neurons compared to birds from Colorado, which provides at least one explanation for superior spatial memory performance (Pravosudov and Clayton, 2002). In contrast to the study of black-capped chickadees, changes in spatial memory in mountain chickadees (*Poecile gambeli*) were not associated with changes in the hippocampal volume and the total neuron number in the hippocampus (Pravosudov and Clayton, 2001; Pravosudov et al., 2002a), a result which suggests that enlarged hippocampal volume with more neurons cannot be the only explanation for enhanced spatial memory performance. However, experiments with mountain chickadees demonstrated that birds with enhanced spatial memory performance had significantly elevated baseline levels of corticosterone (Pravosudov et al., 2001), which suggests that corticosterone may be mediating memory performance even in the absence of any

volumetric changes in the hippocampus. For these reasons, we would predict that Alaskan chickadees might have constantly higher baseline levels of corticosterone than the Colorado birds, irrespective of current environmental conditions and in addition to enlarged hippocampus with more neurons.

Studies by Holberton and Able, (2000) have shown that for some species, at least, birds wintering in more extreme and less predictable environments have similar baseline levels of corticosterone to those living in more favorable environments, but respond to acute stress by elevating their plasma corticosterone to significantly higher levels compared to conspecifics wintering in more favorable environmental conditions. Holberton and Able (2000) suggested that the stronger adrenocortical stress response might enable these birds to make quicker physiological and behavioral adjustments in response to sudden changes in the environment. Again, the question arises whether such population differences in the magnitude of adrenocortical stress response are simply a result of current local environmental conditions. Taken together, all of these results suggest that environmental conditions might affect baseline levels of corticosterone and/or the magnitude of the adrenocortical response to acute stress.

In this study, we addressed two main questions: (1) do black-capped chickadees from Alaska have higher baseline levels of corticosterone than chickadees from Colorado when maintained under the same experimental conditions, and (2) do black-capped chickadees from Alaska show a stronger adrenocortical response to acute stress and reach higher maximum levels of corticosterone than chickadees from Colorado when maintained under the same experimental conditions. We specifically chose to do the comparisons under identical conditions to avoid confounding effects of local environments and to establish if the differences in cache retrieval efficiency and spatial memory found under identical laboratory conditions could be explained by the differences in baseline corticosterone levels or in the magnitude of adrenocortical stress response.

2. Materials and methods

In October of 2000, 15 black-capped chickadees were caught around Anchorage, Alaska (61°10'N, 150°01'W) and 12 black-capped chickadees were

caught around Windsor, Colorado (40°35'N, 105°08'W) using mist nets near the feeders. Within a week after capture, all birds were transported to the University of California in Davis and placed individually in wire-mesh cages (60×42×60 cm). All birds were maintained on a 8:16-h light:dark cycle at a constant 20 °C temperature. Birds from Alaska and Colorado were kept in the same room separated only by a testing chamber and fed with a mixture of shelled sunflower seeds, crushed peanuts and mealworms, and given water with vitamins *ad libitum*.

After all birds were maintained in captivity for 45 days, we tested their memory performance in a cache recovery task and subsequently in three versions of a one-trial associative learning task (Pravosudov and Clayton 2002). After the behavioral experiments (approx. 3 months since bringing the birds into captivity), we collected blood samples. During the entire time, both Alaska and Colorado chickadees have been maintained in identical conditions (see Pravosudov and Clayton, 2002 for complete description). We collected four blood samples from a brachial vein of each bird, one bird at a time. Each bird was captured inside its cage and removed into a different room. The first sample was collected within 3 min of opening the cage, the second sample at 5 min, the third sample at 20 min, and the fourth sample was collected at 50 min after entering the cage. This is a standard procedure for testing adrenocortical stress response in a small bird with a body mass of less than 15 g (e.g. Wingfield et al., 1995). The total amount of blood collected during the stress series did not exceed 1.5% of bird body mass, which should not cause an additional physiological stress to birds. All chickadees were held in cloth bags between sample collections. We used samples collected within 3 min of entering the cage to determine baseline levels of corticosterone because it has been shown that corticosterone levels usually do not start to elevate until 3 min after capture (Wingfield et al., 1982; Kitaysky et al., 1999; Pravosudov et al., 2001, 2002b, see results). Birds from both groups were bled at the same time during the daylight active hours of the day (on average approx. 1300 for both groups; Mann–Whitney test, $n_1 = 14$, $n_2 = 10$, $U = 67$, $P = 0.86$). We were able to use 14 chickadees from Alaska (nine males and five females) and 10 chickadees from Colorado (seven males and three females) for corticosterone analyses. We collected blood in

Table 1

Effects of the population, body mass, wing length, sex and time of day on baseline and the maximum levels of corticosterone reached during the stress response in black-capped chickadees

| Parameter | <i>d.f.</i> | Baseline CORT | | Maximum CORT | |
|------------------|-------------|---------------|----------|--------------|----------|
| | | <i>F</i> | <i>P</i> | <i>F</i> | <i>P</i> |
| Body mass | 1,16 | 9.52 | <0.01 | 0.68 | 0.42 |
| Wing length | 1,16 | 1.36 | 0.26 | 0.28 | 0.60 |
| Time of day | 1,16 | 0.01 | 0.94 | 0.17 | 0.68 |
| Population | 1,16 | 0.18 | 0.68 | 0.02 | 0.88 |
| Sex | 1,16 | 0.12 | 0.73 | 0.27 | 0.61 |
| Population × Sex | 1,16 | 5.32 | 0.03 | 0.27 | 0.61 |

heparinized capillary tubes and then emptied them into 0.3 ml vials that were kept on ice. All samples were centrifuged within 2 h of blood collection, the collected plasma samples were frozen at -20°C and then shipped in dry ice to University of Washington for radioimmunoassay analyses. After collecting blood samples, all birds were killed and their sex was determined.

We measured concentrations of corticosterone after extraction of 5–20 μl samples in dichloromethane. Recovery values of the extraction averaged 89.9% (range 81–100%). To avoid an inter-assay variation we analyzed all samples during a single assay. An intra-assay variance was 4% and sensitivity of the analysis was 7.8 pg/tube. For details of radioimmunoassay analyses see Wingfield and Farner (1975) and Wingfield et al. (1992).

We used an ANCOVA to compare baseline levels of corticosterone between the two population groups and to assess any potential affects of sex, time of day, size, and body mass. We used repeated measures ANOVA (PROC MIXED; SAS Institute, 1994) to compare the adrenocortical stress response including all four samples taken within 50 min of entering the cage. All assumptions of statistical tests were upheld and the significance level was set at 0.05. For the main non-significant results we performed a power analysis. For the power analyses, we used previous work on chickadees which detected a significant increase in corticosterone levels of approximately 75% as a result of manipulations in food supply (Pravosudov et al., 2001). Thus, we calculated the statistical power to detect a 75% difference.

3. Results

3.1. Baseline corticosterone levels

In this study, corticosterone levels did not increase significantly in response to handling within 0–3 min interval after capture (regression analysis of corticosterone concentration on time since entering the cage, $F_{1,21} = 1.24$, $P = 0.28$); thus demonstrating that samples collected within 3 min of entering the cage represent baseline values.

There were no significant differences in baseline plasma corticosterone levels between chickadees from Alaska and Colorado (Table 1 and Fig. 1) and this result appears to be fairly robust (statistical power > 0.7). Time of sampling had no significant effect on the baseline corticosterone levels (Table 1). There were no statistically significant differences between males and females (Table 1) but there was a significant interaction between sex and population: Alaskan males tended to have lower baseline levels of corticosterone than females (Fig. 2), whereas in Colorado birds this tendency was reversed (Fig. 3), but none of the individual differences were statistically significant. Relative body mass (=energy reserves) analytically controlled for wing length (which by itself had no significant effect, Table 1) in the analysis was negatively and significantly related to the baseline levels of corticosterone: heavier birds tended to have lower plasma corticosterone levels compared to lighter birds (Fig. 4 and Table 1).

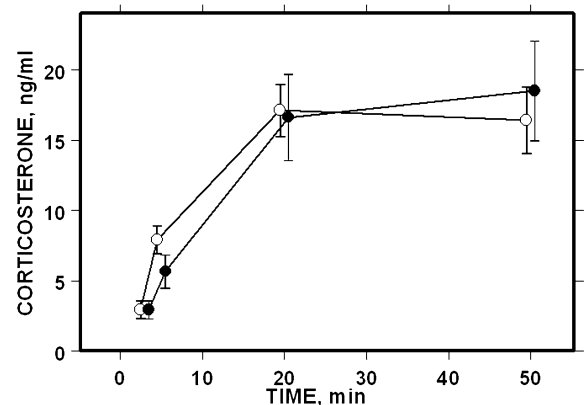


Fig. 1. Adrenocortical response to acute stress (mean and S.E.) in black-capped chickadees from Alaska \circ (open circle) and Colorado \bullet (filled circle) at 0–3, 5, 20, and 50 min after opening the cage. The data points are slightly offset from the time scale to improve the clarity of the data.

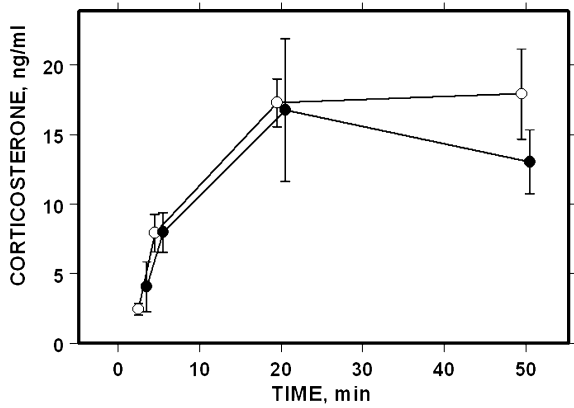


Fig. 2. Adrenocortical response to acute stress (mean and S.E.) in males (○) and females (●) black-capped chickadees from Alaska at 0–3, 5, 20, and 50 min after opening the cage. The data points are slightly offset from the time scale to improve the clarity of the data.

The effect of body mass persisted whether wing length was included in the analysis or not.

3.2. Adrenocortical stress response

Birds from Alaska and Colorado did not differ significantly in the adrenocortical response to acute stress (Table 2 and Fig. 1). Chickadees from both groups increased their levels of corticosterone significantly in response to acute stress (Table 2 and Fig. 1) but analyses of independent contrasts

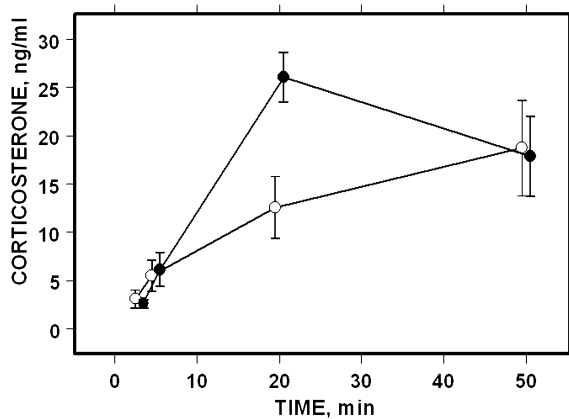


Fig. 3. Adrenocortical response to acute stress (mean and S.E.) in males (○) and females (●) black-capped chickadees from Colorado at 0–3, 5, 20, and 50 min after opening the cage. The data points are slightly offset from the time scale to improve the clarity of the data.

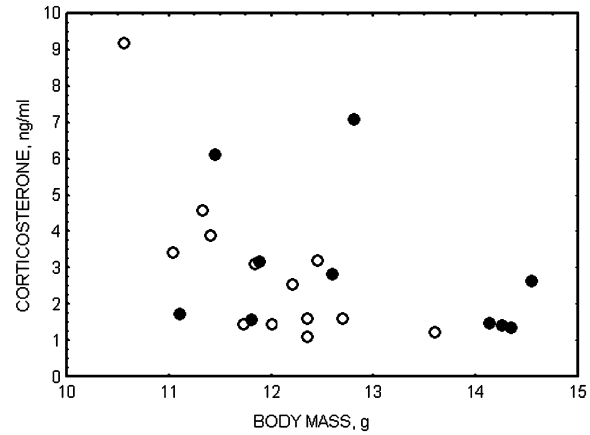


Fig. 4. The relationship between body mass and baseline levels of corticosterone in Alaska (○) and Colorado (●) black-capped chickadees.

revealed that the increase was significant only up to 20 min after inducing acute stress (all P 's < 0.05), whereas there were no significant differences in corticosterone levels between 20 and 50 min since entering the cage ($P=0.39$, Fig. 1). An interaction between population and time since inducing stress was not statistically significant suggesting that both Alaskan and Colorado chickadees had similar adrenocortical responses to acute stress. Consistent with these results, we also found no statistically significant differences in maximum reached levels of corticosterone between the two groups of chickadees (Table 1). The statistical power to detect a 50% and a 75% difference was 0.74 and 0.97, respectively, which is fairly robust. Relative body mass, time of day and sex had no significant effect on maximum reached levels of corticosterone (Table 1).

There were no significant differences between the sexes in their adrenocortical response to acute

Table 2

Effects of the population, time since capture, and sex on the entire adrenocortical stress response in black-capped chickadees

| Parameter | d.f. | F | P |
|-------------------------|------|-------|--------|
| Population | 1,20 | 0.06 | 0.81 |
| Time | 3,56 | 27.31 | <0.001 |
| Sex | 1,20 | 0.33 | 0.57 |
| Population × Sex | 1,20 | 0.79 | 0.38 |
| Time × Sex | 3,56 | 3.73 | 0.02 |
| Time × Population | 3,56 | 0.86 | 0.47 |
| Population × Time × Sex | 3,56 | 2.20 | 0.09 |

stress (Table 2) and an interaction between population group and sex was also not significant (Table 2). An interaction between time since inducing acute stress and sex, however, was statistically significant (Table 2). Independent contrasts analyses revealed that females from Colorado reached significantly higher levels of corticosterone by 20 min since inducing stress compared to Colorado males ($P < 0.01$, Fig. 3), whereas there were no significant differences between the sexes in Alaska chickadees (Fig. 2).

4. Discussion

Our results demonstrate that black-capped chickadees from Alaska and Colorado that are maintained in identical conditions do not differ in their baseline levels of corticosterone or in their adrenocortical response to acute stress. There was a negative relationship between relative body mass and baseline corticosterone level (while no significant relationship between relative body mass and maximum reached levels of corticosterone during a response to acute stress). The only population difference was seen in a population \times time \times sex interaction in which Colorado females reached significantly higher levels of corticosterone by 20 min after inducing stress than Colorado males whereas there were no significant differences between the sexes for Alaskan birds. The statistical power of the main tests was fairly robust minimizing a possibility that we failed to detect a significant difference because of insufficient sample size.

Our results suggest that the differences in food caching behavior and performance on spatial memory tasks between northern and southern black-capped chickadees reported in Pravosudov and Clayton (2002) were not influenced by either differences in baseline corticosterone levels or in the magnitude of adrenocortical response to acute stress. This result is particularly intriguing in view of our previous demonstration that temporal changes in spatial memory performance in mountain chickadees were correlated with differences in baseline levels of corticosterone; i.e. birds maintained on limited and unpredictable food schedule had significantly elevated baseline levels of corticosterone and they demonstrated better performance on spatial memory tasks when compared to the birds maintained on unlimited food schedule (Pravosudov and Clayton, 2001; Pravosudov et al., 2001). Another important detail of the moun-

tain chickadee study was that these changes in memory performance and baseline corticosterone levels were not accompanied by differences in volume of the hippocampus or total number of hippocampal neurons (Pravosudov et al., 2002a).

One way of interpreting the difference between the two studies is to suggest that enhanced performance on tasks that require a highly accurate spatial memory, such as efficient cache recovery, might be mediated in the short term by increases in baseline levels of corticosterone, whereas in the long term they might be mediated by more permanent neuroanatomical changes such as an increase in the total number of neurons and volume of specific brain regions. Thus, for birds that constantly live in harsher environments where food supply is both limited and unpredictable over long periods of time, as is the case with black-capped chickadees in Alaska (vs. Colorado), one might argue that the evolutionary selection pressure on caching intensity, cache retrieval efficiency and spatial memory accuracy is greater than for those living in milder conditions, and that under such conditions birds may develop larger hippocampal volumes with more neurons to support the increased cognitive demands (Pravosudov and Clayton, 2002).

However, because the two studies were conducted on two different species, the possibility remains that the results of these studies could be attributed to a difference between the two species in the way they respond to these stressors. This explanation seems unlikely as black-capped and mountain chickadees are closely related species that are biologically very similar (Harrap and Quinn, 1995). Another alternative explanation is that although there were no significant differences in total corticosterone levels, there could still be differences between experimental birds in the levels of corticosterone binding proteins or in the number of corticosterone receptors in the hippocampal formation (Breuner and Orchinik, 2002). Future studies will be able to test this alternative explanation while the current study only concludes that total corticosterone levels could not explain the differences found in caching behavior and in spatial memory between the two populations of black-capped chickadees.

It is well known that birds respond to temporal changes in the environment by elevating their levels of corticosterone (Wingfield et al., 1998; Silverin, 1998) and that birds temporarily living

in harsher conditions show a stronger response to acute stress (Holberton and Able, 2000). Our study suggests that these differences are probably triggered on a temporal basis by local environmental conditions and, in the absence of environmental differences, there may often be no permanent changes in adrenocortical activity. More data on other species and on levels of corticosterone binding proteins, however, would be necessary to fully verify this claim (Breuner and Orchinik, 2002).

Many studies have found that body condition correlates negatively with the strength of the adrenocortical response to stress (e.g. Wingfield et al., 1998). Contrary to these findings, we did not detect any significant effects of relative body mass on maximum levels of corticosterone reached during the response to acute stress. However, we found that birds with relatively smaller energy reserves had significantly higher baseline levels of corticosterone than birds with relatively large energy reserves. Whereas, it appears that mainly one bird with lowest body mass and highest corticosterone levels (Fig. 3) could have influenced statistical significance of this relationship, removing this point from the analysis did not change the results. All experimental birds were given sufficient food to maintain desired levels of body fat so the variance in body fat levels was likely due to individual strategies (caused by different perception of food supply, predation risk, etc.). Perhaps birds with low energy reserves maintained slightly higher baseline levels of corticosterone to be able to quickly restore energy balance if conditions were to change suddenly.

Supporting our previous studies on mountain chickadees (Pravosudov et al., 2001, 2002b) we found that female black-capped chickadees from Colorado elevate their corticosterone levels significantly faster than males whilst Alaskan birds did not show significant differences between males and females. It is not clear why females from more southern areas should respond more strongly to acute stress than males, as it is not clear why females in general would have a stronger adrenocortical stress response. We have speculated earlier (Pravosudov et al., 2001, 2002b) that parid females are always in more subordinate social status than males within winter groups and as a result their environment may be more unpredictable than that of males. If that is true, then it may be beneficial for females to have a stronger adrenocortical response to stress. It is also possible that

in southern populations the difference in the predictability of the environment between males and females could be more drastic. In northern populations, the environment could be more energetically challenging during the winter (shorter day length, lower ambient temperature, more snow cover, etc.) so that the difference between males and females may be smaller, resulting in males and females responding equally to acute stress. In our experiment, all birds were maintained in identical conditions so the differences found between Colorado males and females cannot be due to differences in their current environment, but the extent to which these sex differences are heritable or result from differences between sexes in their previous environmental exposure remain to be tested experimentally.

In conclusion, our results suggested that corticosterone was not involved in mediating the differences in food caching and spatial memory detected between the two populations living under constantly different environmental conditions. Our study attempted to isolate the comparison of adrenocortical stress response from confounding effects of local environmental conditions and showed that significant differences in behavior found under identical laboratory conditions were not accompanied by significant differences in baseline corticosterone levels or in magnitude of adrenocortical stress response. These results suggest that many population differences in either baseline levels of corticosterone or in magnitude of adrenocortical stress response commonly reported in literature could be mainly due to differences in local environmental conditions and not due to permanent physiological differences between the populations. Certainly, many more comparisons of different species need to be made to verify our suggestion. As our experiments were performed with captive birds, it is possible that captivity had an effect on adrenocortical activity of experimental birds (Marra et al., 1995; Romero and Wingfield, 1999). But because we have tested birds of the same species from different populations and both groups spent considerable amount of time in identical laboratory environment (3 months), it seems unlikely that our results were confounded by possible differences between the populations in a way they adjust to captive conditions. Most importantly, we did find the differences in caching behavior and in spatial memory performance between the two groups of chickadees (Pravosudov and Clay-

ton, 2002) and this study demonstrated that these differences were not associated with differences in either baseline corticosterone levels or in the magnitude of adrenocortical stress response under the same conditions.

Clearly we need more data on adrenocortical activity of animals of the same species but from different populations under natural and controlled identical conditions to better understand the evolution of adrenocortical responses to stress, but this study emphasizes the importance of comparing the role of ecological and phylogenetic factors and the interplay between them. Whereas this and many other studies only address total levels of plasma corticosterone, to fully understand the evolution of adrenocortical function we also need to start measuring levels of corticosterone binding proteins and tissue receptor numbers (Breuner and Orchinik, 2002).

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