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A comparison of the adrenocortical responses to acute stress in cardueline finches from the Tibetan Plateau, Arctic Alaska and lowland Western North America

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Abstract In many avian species, it has been well demonstrated that individuals attempting to breed in harsh or unpredictable environments always express reduced adrenocortical responses to acute stress, whereas those breeding in more benign or predictable environments may show more robust responses. However, fewer studies have focused on comparing closely related species that express similar behavioral traits (e.g., territorial behavior, mating system, nestling behavior) and ecological traits (e.g., habitats, food resources) among different breeding environments. In closely related taxa, we hypothesized that those species breeding in benign environments would show greater adrenocortical responses to acute stress compared with their congeners breeding in harsh environments. In this study, we examined seasonal and sex differences in baseline and stress-induced plasma corticosterone (CORT) levels in four cardueline finch species from Arctic Alaska (reanalysis of previously published data from the Common Redpoll, *Carduelis flammea*, at high latitude habitats), the Tibetan Plateau (Twite, *C. flavirostris*, from high altitude, mid-latitude habitats), and western North America (American Goldfinch, *C. tristis*, and Pine Siskin, *C. pinus*, from mid-latitude and low altitude habitats). Our results showed that (1) Twites had lowered adrenocortical responses during the pre-basic molt stage than the early breeding stage, both sexes of American Goldfinch and Pine Siskin showed similar patterns of adrenocortical responses between the early breeding and the late breeding stages, whereas Common Redpolls expressed significantly lowered adrenocortical responses during the late breeding stage; and (2) unexpectedly, there were no significant differences in baseline or stress-induced CORT (maximal CORT, total integrated and corrected integrated CORT levels) levels among Twites, American Goldfinches, Pine Siskins, and Common Redpolls during the early breeding stage or among American Goldfinches, Pine Siskins and Common Redpolls during the late breeding stage. This similar pattern in adrenocortical responses may reflect the stability of physiological functions of CORT during long-term evolutionary adaptation. It also provides us with an opportunity to understand the potential effects of phylogenetic relationships on the modulation of adrenocortical responses to acute stress in free-living birds. Whether phylogenetic effects are a common or casual phenomenon remains to be determined in other closely related taxa.

Keywords Adrenocortical response · Corticosterone · Cardueline finch · Arctic Alaska · Tibetan plateau

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Zusammenfassung

Vergleich der adrenokortikalen Reaktion auf akuten Stress bei Cardueliden der Tibetischen Hochebene, des arktischen Alaskas und des westlichen Tieflands Nordamerikas

Von vielen Vogelarten ist bekannt, dass Individuen, die in rauen oder unvorhersagbaren Lebensräumen brüten, auf akuten Stress immer mit einer geringeren adrenokortikalen Reaktion antworten als Individuen, die in einer günstigeren oder vorhersehbaren Umwelt brüten und welche stärkere Reaktionen zeigen können. Allerdings gibt es nur wenige zwischen verschiedenen Bruthabitaten vergleichende Untersuchungen zu nahe verwandten Arten mit ähnlichem Verhalten (z. B. Territorialverhalten, Paarungssysteme, Nestlingsverhalten) und ökologischen Bedürfnissen (z. B. Lebensräume, Nahrungsressourcen). Unsere Hypothese für nah verwandte Taxa war, dass Arten, die in einer günstigeren Umwelt brüten, eine stärkere adrenokortikale Reaktion auf akuten Stress zeigen, als deren Gattungsgenossen, die in rauen Lebensräumen brüten. In dieser Studie wurden saisonale und geschlechtsspezifische Unterschiede in den Corticosteron-Plasmawerten (CORT) in Ruhe und unter Stress bei vier Carduelidenarten des arktischen Alaskas (erneute Auswertung bereits veröffentlichter Daten vom Birkenzeisig *Carduelis flammea* aus Habitaten höherer Breiten), der Tibetischen Hochebene (Berghänfling *C. flavirostris* aus Hochgebirgshabitaten mittlerer Breiten) und des westlichen Nordamerikas (Goldzeisig *C. tristis* und Fichtenzeisig *C. pinus* aus Tieflandhabitaten mittlerer Breiten) untersucht. Die Ergebnisse zeigten, dass (1) Berghänflinge während der Postnuptialmauser eine gegenüber der frühen Brutphase verminderte adrenokortikale Reaktion aufwiesen. Bei Goldzeisigen und Fichtenzeisigen zeigten beide Geschlechter zu Beginn und gegen Ende der Brutzeit ähnliche Muster in der adrenokortikalen Reaktion, während Birkenzeisige in der späten Brutphase eine signifikant verminderte adrenokortikale Reaktion aufwiesen. (2) Anders als erwartet gab es keine signifikanten Unterschiede in den CORT-Werten (CORT-Höchstwert, Gesamtwert und korrigierter Wert) in Ruhe und unter Stress zwischen Berghänfling, Goldzeisig, Fichtenzeisig und Birkenzeisig zu Beginn der Brutzeit bzw. zwischen Goldzeisig, Fichtenzeisig und Birkenzeisig gegen Ende der Brutzeit. Dieses ähnliche Muster in der adrenokortikalen Reaktion spiegelt möglicherweise die Stabilität der physiologischen Funktionen von CORT in der langfristigen evolutiven Anpassung wieder. Außerdem stellt es eine Gelegenheit dar, die möglichen Auswirkungen phylogenetischer Verwandtschaft auf die Modulation adrenokortikaler Reaktionen wildlebender Vögel auf akuten Stress zu verstehen. Ob phylogenetische Effekte ein häufiges oder gelegentliches Phänomen darstellen, muss noch bei anderen nahe verwandten Taxa geklärt werden.

Introduction

The adrenocortical response to stress may help an animal to cope with various environmental perturbations by activating the hypothalamo-pituitary-adrenal (HPA) axis (Wingfield et al. 1998; Sapolsky et al. 2000; Romero 2002; Wingfield 2003). All avian species studied to date can rapidly increase the plasma levels of corticosterone (CORT, the major avian glucocorticoid) in response to acute stressors (Wingfield et al. 1992, 1998; Holberton and Able 2000; Romero 2002; Romero et al. 2006). The short-term (acute) increase in CORT levels can enhance survival by suppressing 'unnecessary' behavioral and physiological functions and activating immediate lifesaving behavioral patterns (i.e. temporary emergency behavior; Wingfield and Ramenofsky 1999; Wingfield and Hunt 2002). However, prolonged high concentration of CORT circulating in blood can lead to a multitude of deleterious effects, including reproductive failure, growth inhibition, immune suppression and neuron damage (Sapolsky 1992; Wingfield et al. 1995; Silverin 1998; Sapolsky et al. 2000; Zhang et al. 2011). To avoid this conflict, a balance must be struck between short-term effects of CORT to promote survival and then moderating CORT secretion to prevent deleterious effects.

Most avian species studied to date show a seasonal modulation of CORT release (both baseline CORT and stress-induced CORT) during different life history stages (Romero 2002). The higher baseline CORT level during nonbreeding always reflects poor energetic conditions, and the reduced stress-induced CORT level during pre-basic molt stage in passerines is necessary for producing high-quality feathers (Romero et al. 2005; Bonier et al. 2009). During the breeding stage, the reduced stress-induced CORT level is always accompanied by the sex providing more parental care (Wingfield et al. 1995; O'Reilly and Wingfield 2001; Meddle et al. 2003; Holberton and Wingfield 2003; Wilson and Holberton 2004; Bókony et al. 2009).

Previous studies indicated that different breeding environments can lead to differences in sensitivity and plasticity of the regulation of adrenocortical response to stress. Individuals attempting to breed in harsh or unpredictable environments (with a short nesting season and limited reproductive opportunities) always express reduced adrenocortical responses, which may allow them to obtain maximal reproductive success, whereas those breeding in more benign or predictable environments (with a long nesting season and more reproductive opportunities) may show robust adrenocortical responses (Wingfield et al. 1992, 1994a, b; Wingfield and Hunt 2002; Boonstra 2004; but see Wingfield et al. 1995). For example, compared with temperate-zone populations, Arctic and high altitude

breeding species expressed reduced adrenocortical responses during the breeding stage (Silverin and Wingfield 1998; Pereyra and Wingfield 2003; Wilson and Holberton 2004; but see Li et al. 2011). This reduced adrenocortical response can increase fitness by enhancing reproductive success (Wingfield 1994; Bókony et al. 2009; Hau et al. 2010).

The Qinghai-Tibetan Plateau (the Tibetan Plateau) is the largest and highest plateau in the world with an average altitude of 4,500 m above sea level (Zhang et al. 2002). Similar to Arctic Alaska, the Tibetan Plateau has treeless expanses with low amounts of grass- and forb-based primary productivity, and simplified food webs (Richardson et al. 2003). Therefore, those species breeding both in the Tibetan Plateau and in Arctic Alaska are characterized by shorter breeding seasons and unpredictable events such as inclement weather and patchy food (Astheimer et al. 1995; Wingfield and Kitaysky 2002; Wingfield et al. 2011). In contrast, their congeners breeding in low altitude or mid-latitude areas may have longer breeding seasons and more benign or predictable environments (e.g., in many temperate regions). In phylogenetic and evolutionary perspective, closely related species can express similar behavioral traits (e.g., territorial behavior, mating system, nursing behavior) and ecological traits (e.g., habitats, food resources). Comparing the adrenocortical responses of closely related species that breed in the same habitats or at different latitude or altitude would be useful to illustrate the phylogenetic effects in plasticity (or stability) of the hormone mechanisms (Wingfield et al. 1997).

In this study, we examined the adrenocortical responses in four species of cardueline finches breeding in Arctic Alaska (Common Redpoll, *Cardulis flammea*, at high latitude habitats), the Tibetan Plateau (Twite, *C. flavirostris*, from high altitude and mid-latitude habitats), and western North America (American Goldfinch, *C. tristis*, and Pine Siskin, *C. pinus*, from mid-latitude and low altitude habitats). We then compared baseline CORT and stress-induced

CORT levels during the early breeding and the late breeding stages. We hypothesized that benign environments (i.e. western North America) breeders would show more robust adrenocortical responses to acute stress whereas harsh environments (both Alaska Arctic and the Tibetan Plateau) breeders would show reduced adrenocortical responses. This type of investigation may also allow us to assess the phylogenetic effects on modulation of adrenocortical responses to acute stress.

Materials and methods

Species, study sites

Four species of cardueline finches (Common Redpoll, Twite, and Pine Siskin and American Goldfinch) from Arctic Alaska, the Tibetan Plateau, and western North America, respectively, were used in this study. Free-living birds were captured opportunistically by Japanese mist nets or in Potter traps baited with seeds during different life history stages at high latitude (Arctic Alaska), high altitude (the Tibetan Plateau) or mid-latitude and low altitude (western North America, see Table 1).

Capture stress protocol and sampling

All birds were sampled using a standardized capture stress protocol (Wingfield et al. 1992; Li et al. 2008). Immediately after capture (within 2–3 min), approximately 30 μl of blood was removed for baseline CORT level. Then, birds were placed in opaque cloth bags and subsequent samples were collected at 5 min, 20 min and 60 min (10 min, 30 min and 60 min for Twites) after capture to create the plasma profile of acute CORT response to unpredictable events.

Blood was obtained from the alar vein in the wing by piercing the vessel with a 26-gauge needle and collecting

Table 1 Time, sites, and sample sizes for the four cardueline finch species studied

Species	Field sites	Time of sampling	Life history stages	Number of individual
Twite	Wenquan, the Tibetan Plateau (35.40°N, 99.43°E, elevation 3,946 m)	4–5 May 2006	Early breeding	10 (male and female)
	Haibei, the Tibetan Plateau, China (37.62°N, 101.32°E, elevation 3,200 m)	2 Aug–1 Sep 2006	Pre-basic molt	22 (male and female)
American Goldfinch	Duvall, Washington (47.5°N,122°W, elevation 50 m)	6–8 May 1997	Early breeding	14 (male 11, female 3)
		19 Jun–5 Jul 1996	Late breeding	17 (male 10, female 6)
Pine Siskin	Duvall, Washington (47.5°N, 122°W, elevation 50 m)	8–10 Apr 1997	Early breeding	15 (male 9, female 6)
		19–21 Jun 1996	Late breeding	20 (male 10, female 10)
Common Redpoll	Toolik Lake, Alaska (68.63°N, 149.6° W, elevation 720 m)	31 May–3 Jun 1999	Early breeding	8 (male 6, female 2)
		3–27 Jun 1992	Late breeding	11 (male 7, female 4)

blood in heparinized microhematocrit capillary tubes. Blood samples were stored on ice until centrifuged (855g for 10 min) within 3–4 h. Plasma was collected and stored at −20°C, and transported to the University of Washington for the assay of CORT.

After sampling, each bird was weighed to the nearest 0.1 g and scored for furcular fat on a semi-quantitative scale of 0 (no fat visible) to 5 (bulging fat deposit; Wingfield and Farner 1978; Biebach et al. 1986). Individuals were measured for wing length, culmen, tarsus length and width and height of cloacal protuberance (a sperm storage and copulation organ) and also examined for presence or absence of brood patch and stage of molt (for Twites). Sex identification for Common Redpolls, American Goldfinches and Pine Siskins was determined by a combination of plumage color and body size or unilateral laparotomy. Sex was not determined for Twites. On completion of sampling, all birds were marked for identification with a metal band (either from the U.S. Fish and Wildlife Service or from the National Bird Banding Center of China) and then released.

Radioimmunoassay of corticosterone

The measurement of CORT levels followed the methods of Wingfield et al. (1992) and O'Reilly and Wingfield (2003). Briefly, 10–20 μl of plasma was allowed to equilibrate overnight with 2,000 cpm of ^{3}H-CORT for determination of individual recoveries. Samples were extracted in 4 ml of freshly redistilled dichloromethane, dried under nitrogen and re-suspended in 550 μl phosphate-buffered saline with 1% gelatin. Samples were assayed in duplicate, and assay values were corrected for plasma volume and individual recoveries after extraction. Inter-assay coefficient of variation was 9.1% and intra-assay variation ranged between 2.7 and 6.9%. Recoveries ranged from 85.7 to 93.6%.

Data analysis

The maximal CORT level was calculated as the maximum concentration of each individual achieved during the capture-handling protocol. The total integrated CORT (the amount of CORT secreted over the sample collection period including both the baseline CORT and the extra CORT secretion in response to capture stress) and corrected integrated CORT (the amount of extra CORT secretion in response to capture stress minus the baseline) were determined as the area under curve (maximal CORT level) calculated by trapezoid rule using GraphPad Prism 5.0 (Cockrem and Silverin 2002; Adams et al. 2005). Comparisons of body mass, fat score, CORT (baseline, maximum, total and corrected integrated levels) between sexes or stages were made by independent samples t test,

and among species were made by one-way ANOVA. Differences between pairs of means were identified post hoc tests using LSD tests. Before statistical analysis, we tested for the homogeneity of variances using Levene's test of equality of variances. If variances among data were significantly different, we used Kruskal–Wallis one-way ANOVA or Mann–Whitney U tests to compare the differences among or between groups. All tests were performed using SPSS software. All data are presented as means ± SEM.

Results

Twite

Body condition and baseline CORT

Neither body mass nor fat score of Twites differed between the early breeding and the pre-basic molt stages. Baseline CORT at the pre-basic molt stage was significantly lower than in the early breeding stage (Table 2; Fig. 1a).

Stress-induced CORT levels

Maximal CORT and total integrated CORT levels during the early breeding stage were significantly higher than those in the pre-basic molt stage. However, there was no significant difference in corrected integrated CORT level (Table 2; Fig. 1b, c, d).

American Goldfinch

Body condition and baseline CORT

The body masses of both male and female American Goldfinches were higher during the late breeding stage than those in the early breeding stage. However, neither sexes showed significant changes in fat score and baseline CORT level between the early breeding and the late breeding stages (Table 3; Fig. 2a).

Table 2 Comparisons of body condition and CORT levels between the early breeding and the pre-basic molt stages in Twites *Carduelis flavirostris*

Factors	F	P
Fat score	$F_{1,27} = 4.283$	0.952
Body mass	$F_{1,27} = 0.233$	0.532
Baseline CORT	$F_{1,25} = 2.452$	0.01
Maximal CORT	$F_{1,24} = 2.828$	0.029
Total integrated CORT	$F_{1,26} = 0.016$	0.015
Corrected integrated CORT	$F_{1,23} = 0.038$	0.092

Fig. 1 The baseline CORT (**a**), maximal CORT (**b**), total (**c**) and corrected integrated CORT levels (**d**) of *Twite*, *Carduelis flavirostris* (early breeding: $n = 5$, pre-basic molt: $n = 22$), American Goldfinch, *C. tristis* (*AMGO*, early breeding: $n = 14$, late breeding: $n = 16$), Pine Siskin, *C. pinus* (*PISI*, early breeding: $n = 15$, late breeding: $n = 20$), and Common Redpoll *C. flammea* (*CORE*, early breeding: $n = 8$, late breeding, $n = 12$; late breeding data were previously published and reanalyzed with permission from Wingfield et al. 1994a) during the early and late breeding, or the pre-basic molt stages. *Asterisk* statistically significant difference ($P < 0.05$)
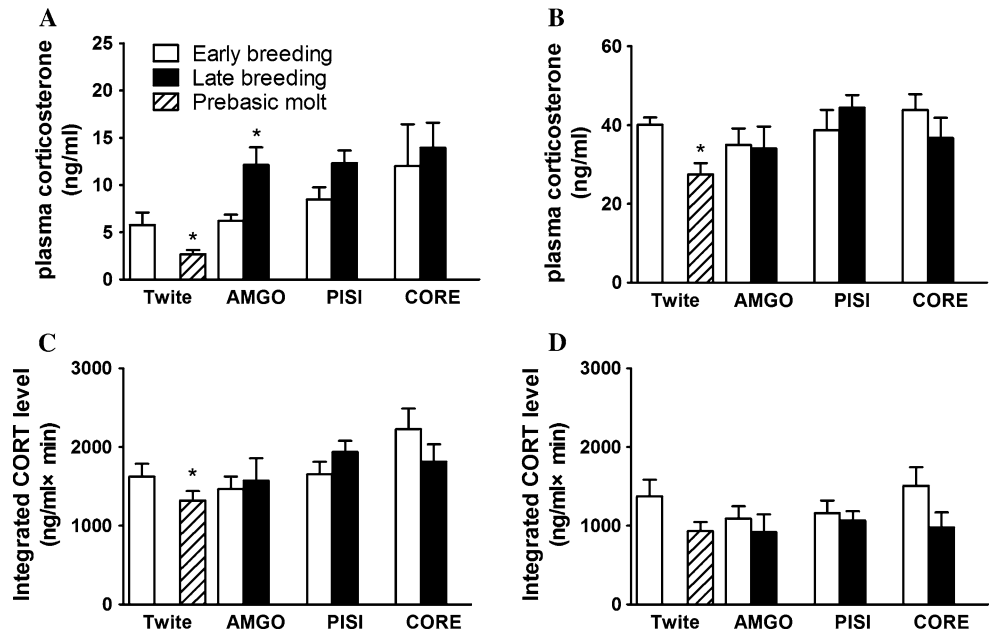

Table 3 Comparisons of body condition and CORT levels between the early and late breeding stages in males and females of American Goldfinch, *Carduelis tristis*, and Pine Siskin, *C. pinus*

Species	Factors	Male		Female	
		F	P	F	P
American Goldfinch	Fat score	$F_{1,19} = 2.073$	0.645	$F_{1,7} = 5.019$	0.197
	Body mass	$F_{1,19} = 0.401$	0.012	$F_{1,7} = 0.088$	<0.001
	Baseline CORT	$F_{1,19} = 4.063$	0.06	$F_{1,7} = 3.707$	0.12
	Maximal CORT	$F_{1,19} = 0.056$	0.722	$F_{1,7} = 1.733$	0.642
	Total integrated CORT	$F_{1,19} = 0.865$	0.525	$F_{1,7} = 1.204$	0.909
	Corrected integrated CORT	$F_{1,19} = 0.656$	0.981	$F_{1,6} = 2.79$	0.075
Pine Siskin	Fat score	$F_{1,17} = 0.164$	0.014	$F_{1,13} = 0.596$	0.075
	Body mass	$F_{1,15} = 0.447$	0.545	$F_{1,13} = 1.459$	0.215
	Baseline CORT	$F_{1,15} = 2.628$	0.023	$F_{1,14} = 0.001$	0.613
	Maximal CORT	$F_{1,17} = 4.822$	0.548	$F_{1,14} = 4.676$	0.124
	Total integrated CORT	$F_{1,15} = 2.662$	0.512	$F_{1,14} = 0.77$	0.289
	Corrected integrated CORT	$F_{1,15} = 0.205$	0.616	$F_{1,14} = 0.481$	0.649

Stress-induced CORT levels

Males and females of American Goldfinches did not show significant differences in maximal CORT, or total and corrected integrated CORT levels between the early breeding and the late breeding stages (Table 3; Fig. 2b–d).

Sex differences in body condition and CORT secretion

Although the sexes did not differ in body mass, fat score, baseline CORT, total integrated CORT or corrected integrated CORT levels during the early breeding stage, males expressed significantly higher maximal CORT level than females (Table 4; Fig. 2). There were no differences between sexes in fat score, baseline CORT, maximal CORT, or total integrated CORT levels during the late

breeding stage. However, females showed significantly higher body mass and lower corrected integrated CORT level than males (Table 4; Fig. 2).

Pine Siskin

Body condition and baseline CORT

Males and females of Pine Siskins did not show changes in body mass during the early breeding and the late breeding stages. Males had more fat during the early breeding stage than the late breeding stage, whereas females did not show significant differences (Table 3). Males had higher baseline CORT levels during the late breeding stage than the early breeding stage, whereas there were no significant differences in females (Table 3; Fig. 2).

Fig. 2 The baseline CORT (**a**), maximal CORT (**b**), total (**c**) and corrected integrated CORT levels (**d**) in female and male of American Goldfinch during the early and late breeding stages (*AMGO*, early breeding, *EB*: female $n = 3$, male $n = 11$; late breeding, *LB*: female $n = 6$, male $n = 10$) and Pine Siskin (*PISI*, early breeding, *EB*: female $n = 6$, male $n = 9$, late breeding, *LB*: female $n = 10$, male $n = 10$). Groups with different *letters* are significantly different from one another ($P < 0.05$)
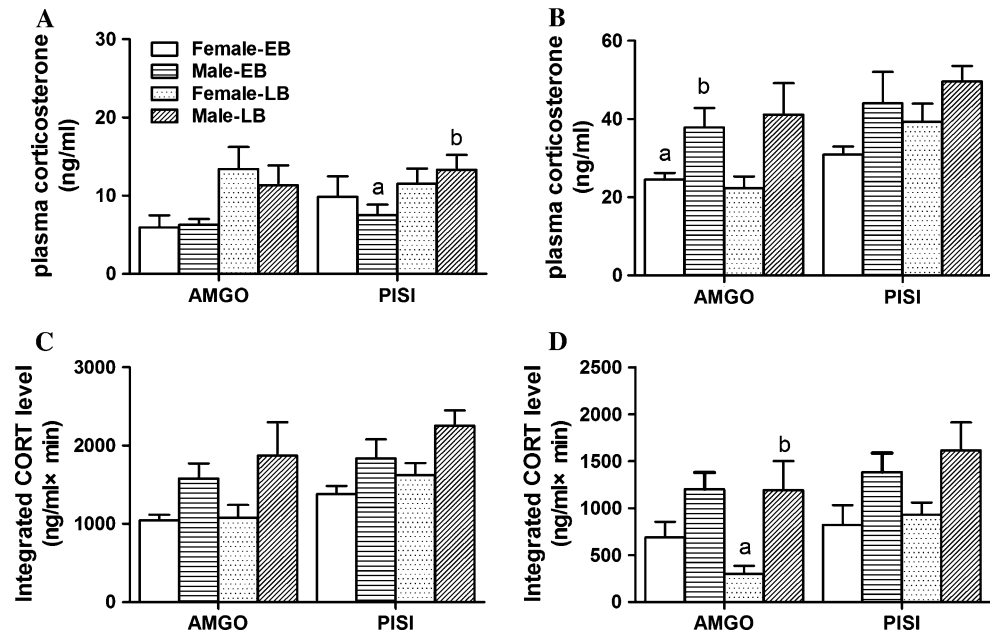
Table 4 Comparisons of body condition and CORT levels between sexes of American Goldfinch and Pine Siskin during the early and late breeding stages

Species	Factors	Early breeding		Late breeding	
		F	P	F	P
American Goldfinch	Fat score	$F_{1,12} = 0.055$	0.577	$F_{1,14} = 3.272$	0.523
	Body mass	$F_{1,12} = 1.2$	0.998	$F_{1,14} = 2.195$	0.001
	Baseline CORT	$F_{1,12} = 0.193$	0.832	$F_{1,14} = 0.02$	0.605
	Maximal CORT	$F_{1,12} = 6.781$	0.028	$F_{1,14} = 1.522$	0.102
	Total integrated CORT	$F_{1,12} = 2.898$	0.191	$F_{1,14} = 1.419$	0.185
	Corrected integrated CORT	$F_{1,12} = 1.391$	0.184	$F_{1,13} = 2.933$	0.086
Pine Siskin	Fat score	$F_{1,12} = 1.174$	0.355	$F_{1,18} = 1.265$	0.001
	Body mass	$F_{1,11} = 0.297$	0.894	$F_{1,17} = 2.507$	0.054
	Baseline CORT	$F_{1,13} = 2.118$	0.402	$F_{1,16} = 0.056$	0.528
	Maximal CORT	$F_{1,13} = 10.329$	0.147	$F_{1,18} = 0.107$	0.111
	Total integrated CORT	$F_{1,13} = 5.817$	0.112	$F_{1,16} = 0.009$	0.085
	Corrected integrated CORT	$F_{1,13} = 0.05$	0.086	$F_{1,16} = 0.053$	0.197

Stress-induced CORT levels

Males and females did not show significant differences in maximal CORT, or total and corrected integrated CORT levels between the early breeding and the late breeding stages (Table 3; Fig. 2).

Sex differences in body condition and CORT secretion

There were no differences in body mass, baseline CORT and maximal CORT levels, or total and corrected integrated CORT levels between the sexes during the early breeding and the late breeding stage (Fig. 2). However, females had a higher fat score than males (Table 4).

Common Redpoll

Body condition and baseline CORT

There were no significant differences in body mass, fat score, and baseline CORT levels in Common Redpolls between the early breeding and the late breeding stages (Table 5, Fig. 1a).

Stress-induced CORT levels (re-analyzed from Wingfield et al. 1994a)

Common Redpolls had significantly higher maximal CORT and total integrated CORT levels during the late breeding

stage than the early breeding stage (Table 5; Fig. 1b, c). However, there were no significant differences in corrected integrated CORT level (Table 5; Fig. 1d).

Sex differences in CORT secretion

There were no significant differences in body mass ($F_{1,7} = 2.841$, $P = 0.99$), fat score ($F_{1,7} = 0.096$, $P = 0.216$), baseline CORT ($F_{1,7} = 8.588$, $P = 0.068$), maximal CORT ($F_{1,7} = 1.636$, $P = 0.356$), or total ($F_{1,7} = 1.435$, $P = 0.306$) and corrected integrated CORT levels ($F_{1,7} = 0.037$, $P = 0.518$) between sexes during the late breeding stage.

Comparison of adrenocortical responses in cardueline finches

Baseline CORT

There were no significant differences in baseline CORT levels during the early breeding stage among Twites, American Goldfinches, Pine Siskins, and Common Redpolls, or during the late breeding stage among American Goldfinches, Pine Siskins, and Common Redpolls (Table 6; Fig. 1a).

Table 5 Comparisons of body condition and CORT levels between the early breeding and the late breeding stages in Common Redpoll *Carduelis flammea*

Factors	F	P
Fat score	$F_{1,16} = 0.539$	0.938
Body mass	$F_{1,15} = 1.472$	0.692
Baseline CORT	$F_{1,16} = 0.965$	0.962
Maximal CORT	$F_{1,16} = 1.333$	0.023
Total integrated CORT	$F_{1,16} = 1.306$	0.046
Corrected integrated CORT	$F_{1,16} = 0.005$	0.123

Table 6 Comparisons of baseline and stress-induced CORT levels among Twite, American Goldfinch, Pine Siskin, and Common Redpoll during the early breeding stage and among American Goldfinch, Pine Siskin, and Common Redpoll during the late breeding stage

Factors	Early breeding		Late breeding	
	F	P	F	P
Baseline CORT	$\chi^2_{3,38} = 1.8$	0.615	$F_{2,42} = 0.237$	0.79
Maximal CORT	$F_{3,38} = 0.574$	0.636	$F_{2,44} = 1.601$	0.213
Total integrated CORT	$F_{3,38} = 2.721$	0.605	$F_{2,44} = 0.803$	0.454
Corrected integrated CORT	$F_{3,38} = 0.986$	0.41	$F_{2,43} = 1.008$	0.373

χ^2 value from Kruska-Wallis ANOVA on ranks

Stress-induced CORT levels

There were no significant differences in maximal CORT, or total and corrected integrated CORT levels during the early breeding stage among Twites, American Goldfinches, Pine Siskins, and Common Redpolls, or during the late breeding stage among American Goldfinches, Pine Siskins, and Common Redpolls (Table 6; Fig. 1b–d).

Discussion

All the cardueline species sampled in this study showed an increase in plasma levels of corticosterone following the standardized acute stress of capture, handling and restraint. Such responses are probably ubiquitous across vertebrates and serve to allow the animal to cope, physiologically and behaviorally, with perturbations of the environment (e.g., Sapolsky et al. 2000; Wingfield and Kitaysky 2002; Wingfield 2003). It is also clear that both baseline and stress-induced levels of glucocorticoids change throughout the year in relation to life history stage, season, gender; etc. (Romero 2002; Wingfield and Sapolsky 2003; Landys et al. 2006). Although there are several hypotheses as to why the adrenocortical response to stress may be modulated in relation to reproductive function (Wingfield and Sapolsky 2003; Bókony et al. 2009; Hau et al. 2010), comparisons of species in different habitats in which phylogeny is controlled will be important to determine adaptive changes in the acute stress response.

Baseline CORT

Although earlier studies by Richardson et al. (2003) and Li et al. (2008) demonstrated there were no differences in baseline CORT between the early breeding and the pre-basic molt stages in snowfinches, *Montifringilla* spp., and Eurasian Tree Sparrows, *Passer montanus* from the Tibetan Plateau, the current results here show that Twites significantly lowered their baseline CORT during the pre-basic molt stage. Most small passerine species undergo a complete feather replacement in the late summer and autumn after the breeding stage, and their baseline CORT levels are often the lowest of the life cycle at this time (Romero and Wingfield 1998; Romero 2002). The down-regulation of CORT release at this period is important to allow production of high quality feathers by promoting the normal amino acid deposition at the site of feather synthesis and inhibiting the protein catabolism (Romero et al. 2005).

Generally, elevated baseline CORT levels during the nonbreeding season are assumed to indicate individuals in challenging energetic conditions or with lower fitness

(Wingfield et al. 1994a; Holberton 1999; Walker et al. 2005; Bonier et al. 2009), but may not necessarily vary with stage of breeding or sex (Wilson and Holberton 2004). Similar to earlier studies, both males and females of arctic breeding American Tree Sparrows, *Spizella arborea*, and White-crowned Sparrows, *Zonotrichia leucophyrys*, showed no significant differences in baseline CORT level between pre-parental and parental stages (Holberton and Wingfield 2003). In this investigation, both sexes of American Goldfinches, female Pine Siskins, and Common Redpolls show no differences in baseline CORT levels between the early and late breeding stages. In contrast, lower baseline CORT levels in pre-breeding male Pine Siskins are concurrent with better energetic condition (greater fat score) during the early breeding stage. Our results support the hypothesis of the negative relationship between baseline CORT level and energetic condition in birds. That is, individuals in poor energetic condition have higher baseline CORT levels (Love et al. 2005). The elevated baseline CORT levels during the late breeding stage with poor energetic condition may contribute to their additional energetic demand of provisioning rapidly growing chicks.

Stress-induced CORT

Similar to the Arctic-breeding Lapland Longspurs, *Calcarius lapponicus*, Snow Buntings, *Plectrophenax nivalis*, and Common Redpolls showing significantly lowered stress-induced CORT levels during the pre-basic molt stage (Romero et al. 1998a, b, c), Twites have significantly lower maximal CORT and total integrated CORT levels during the pre-basic molt stage than in the early breeding stage. Our results are consistent with other passerines studies to date that also show very low levels of stress-induced CORT during their pre-basic molt stage. Because high levels of glucocorticoids tend to increase amino acid deamination and breakdown of protein, very low levels during molt may be important to promote their survival by accelerating the rate and quality of feather growth (Romero et al. 2005). Interestingly, although there were no significant differences when the corrected integrated CORT levels were calculated in Twites between the early breeding and the pre-basic molt stages, our results contrast with previous findings in resident species—snowfinches and Eurasian Tree Sparrow sampled from the Tibetan Plateau showing no significant differences in maximal CORT and total integrated CORT levels (Richardson et al. 2003; Li et al. 2008). As a migratory species, Twites are present on the Tibetan Plateau during the breeding period. Therefore, the peak maximal CORT and total integrated CORT levels being consistent with the increased baseline CORT level may in Twites reflect the challenging energetic conditions

after their long-distance migration during the early breeding stage.

Wilson and Holberton (2004) showed that in male arctic-breeding Yellow Warblers, *Dendroica petechia*, there was a significantly higher stress response during pre-parental stage than those from provisioning stage, whereas temperate zone breeding males expressed no differences while nesting. Similarly, we were unable to find statistically significant differences in maximal CORT, or total and corrected integrated CORT levels in low altitude and mid-latitude carduelinine species (both sexes of Pine Siskin and American Goldfinch) between the early and late breeding stages. In contrast, the Alaska Arctic Common Redpolls showed significantly lowered maximal CORT and total integrated CORT levels during the late breeding stage than the early breeding stage. This reduced stress response, when it appears in breeding period, may be an adaptive strategy to increase their parental care and promote reproductive success as seen in other species breeding at high latitude (Wingfield et al. 1995; Wingfield and Sapolsky 2003).

However, when the degree of parental expenditure by males and females was considered, females had lower adrenocortical responses than males during incubation period possibly related to the females' investment in egg development and incubation (Wilson and Holberton 2004; Meddle et al. 2003). When the sexes were compared, male American Goldfinches had higher maximal CORT levels than females during the early breeding stage (Fig. 2b), and higher corrected integrated CORT levels than females during the late breeding stage (Fig. 2d), whereas there were no significant differences in Pine Siskins and Common Redpolls. The relationship between parental investment and modulation of the adrenocortical response has been documented in many previous studies (Wingfield et al. 1995; O'Reilly and Wingfield 2001; Meddle et al. 2003; Wingfield and Sapolsky 2003). These studies suggest that the sex providing more parental care should have lower stress response than the other sex. Both Pine Siskins and American Goldfinches are socially monogamous, females build their nests and incubate eggs in the early breeding stage, and both sexes feed nestlings and fledglings in the late breeding stage. It is interesting that females of American Goldfinches showed lowered maximal CORT and corrected integrated CORT levels during breeding. Whether the female American Goldfinches show more parental care than males remains to be clarified.

Comparison of adrenocortical responses in carduelinine finches

Our results reveal that high altitude species (Twite) showed lowered baseline CORT and stress-induced

CORT levels during the pre-basic molt than the early breeding stage, which is similar to previous findings in high latitude species (Common Redpoll; Romero et al. 1998c). Both sexes of mid-latitude and low altitude species (American Goldfinch and Pine Siskin) show similar patterns of baseline CORT (except for male Pine Siskins showing significantly lower levels during early breeding stage) and stress-induced CORT levels between the early breeding and the late breeding stages, whereas high latitude species (Common Redpoll) express significantly lowered stress-induced CORT levels during the late breeding stage. Our results provide more evidence for the seasonal modulation of adrenocortical responses in free-living birds. Furthermore, both the baseline CORT and stress-induced CORT levels can vary with sexes and breeding stages as well.

However, when the adrenocortical responses were compared among all four cardueline finch species (in both benign and harsh environments) at species level, unexpectedly, we did not find any significant differences in baseline CORT and stress-induced CORT levels during the early and late breeding stages. Therefore, by controlling for environment effects, we no longer see a latitude effect between early and late breeding stages with baseline CORT levels, but we do see it with maximal CORT and corrected integrated CORT levels. By comparing the phylogeny, we see no effects of environments on adrenocortical responses during the early breeding stage. Although the adrenocortical responses of free-living birds can vary with sex, life history stage, and environments at population level, in this study, we do not see any effects of habitat (both harsh and benign environments) at species level of closely related taxa.

This similar pattern of adrenocortical responses in four cardueline finch species may reflect the stability of physiological functions of CORT during long-term evolutionary adaptation. It also provides us with an opportunity to understand the potential effects of phylogenetic relationships on the modulation of adrenocortical responses to acute stress in free-living birds. Clearly, these species show similar responses to acute stress regardless of habitat, and different patterns of responses from unrelated species. Whether phylogenetic effects are a common or casual phenomenon remains to be determined in other closely related taxa.

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