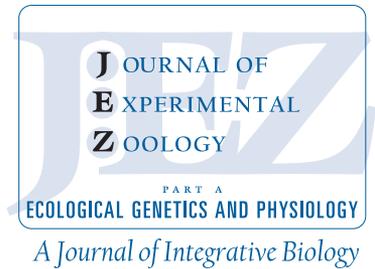


Comparison of Adrenocortical Responses to Acute Stress in Lowland and Highland Eurasian Tree Sparrows (*Passer montanus*): Similar Patterns During the Breeding, But Different During the Prebasic Molt



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ABSTRACT

Previous studies indicate most free-living avian species in both extreme and temperate environments seasonally modulate the adrenocortical responses to acute stress, and those breeding in harsh environments always express reduced adrenocortical responses, which may allow them to obtain maximal reproductive success. However, recent investigations showing a human commensal species, house sparrows (*Passer domesticus*), expressed similar corticosterone (CORT) responses in both benign and harsh environments. In this study, focusing on another human commensal species, Eurasian tree sparrows (*P. montanus*), we examined the adrenocortical response to acute stress in lowland populations, among the early and late breeding, the prebasic molt, and the wintering stages, and compared them with previously published data from populations on the Tibetan Plateau. Our results show: (1) similar to highland Eurasian tree sparrows, lowland populations show no differences in baseline CORT levels among life history stages, and the stress-induced CORT (maximal CORT, total and corrected integrated CORT) levels are lower during the early breeding and the prebasic molt stages than those in the late breeding and the wintering stages; (2) highland Eurasian tree sparrows show stronger adrenocortical responses during the prebasic molt stage than lowland populations, whereas there are no differences between the early and the breeding stages (except for maximal CORT). Our results suggest that Eurasian tree sparrows from both harsh and benign environments have similar patterns of adrenocortical responses in the breeding stage, whereas they are different in the prebasic molt stage. In highland birds, the increased maximal CORT levels during the late breeding and the small increases in adrenocortical responses during the prebasic molt are interesting but remain unexplained. *J. Exp. Zool.* 315:512–519, 2011. © 2011 Wiley Periodicals, Inc.

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In wild vertebrates, acute stress stimulates the hypothalamic–pituitary–adrenal (HPA) axis to release glucocorticoids rapidly, which helps protect individuals from external noxious stimuli (Wingfield et al., '98; Sapolsky et al., 2000; Wingfield and Kitaysky, 2002). In response to environmental perturbations, the rapidly increased concentrations of corticosterone (CORT, the major glucocorticoid in birds) above baseline levels (nonstressed condition) may contribute to survival by suppressing “unnecessary” activities (e.g. suppressing territorial and reproductive behaviors) and promoting “temporary emergency behaviors” (e.g. increased gluconeogenesis and mobilization of fat stores; Wingfield et al., '98; Sapolsky et al., 2000; Wingfield and Hunt, 2002). However, prolonged high levels of CORT can inhibit reproduction, suppress the immune system, and promote protein loss, leading to lower Darwinian fitness (Sapolsky et al., 2000). Thus, individuals would benefit from modulating their adrenocortical responses to acute stress over time to sustain an optimized physiological state and avoid the deleterious effects on fitness.

Different life history stages and breeding environments can lead to differences in the regulation of adrenocortical responses. Recent evidences demonstrate that most free-living avian species in both extreme and temperate environments seasonally modulate the magnitude of CORT responses to acute stress (Romero, 2002; Li et al., 2008). The peak of baseline and/or stress-induced CORT levels may appear in prebreeding or wintering life history stages, whereas the nadir happens during the prebasic molt in most small passerines (Romero, 2002). To date, what induces the seasonal changes of CORT release remains unknown, but there are three major hypotheses explaining this phenomenon: the energy mobilization hypothesis (emphasizing the importance of seasonal energy expenditure), the behavior hypothesis (emphasizing the annual changes of CORT result from animals having different requirements for expressing/not expressing the CORT-mediated behaviors at different times of the year), and the preparative hypothesis (emphasizing the annual changes of CORT serve to modulate the priming of stress pathways during periods with different potential exposures to adverse conditions, e.g. initial stress responses help the organism prepare for subsequent ones; Sapolsky et al., 2000; Romero, 2002; Romero et al., 2006). Furthermore, individuals can modulate their adrenocortical responses on the basis of their physiological state and the precise nature of environmental circumstances (Reneerkens et al., 2002). For example, individuals attempting to breed in harsh or unpredictable environments always express reduced adrenocortical responses, which may allow them to obtain maximal reproductive success, whereas those breeding in more benign or predictable environments may show robust adrenocortical responses (Wingfield et al., '92, '94; Silverin and Wingfield, '98; Wingfield and Hunt, 2002; but see Wingfield et al., '95).

Previous studies indicate that the house sparrow (*Passer domesticus*), a human commensal species, seasonally modulates

adrenocortical responses to acute stress (the highest CORT levels during the breeding and lowest during the prebasic molt stages); however, there were nearly equivalent CORT responses in both harsh and benign environments (Breuner and Orchinik, 2001; Romero et al., 2006; Li et al., 2008). This may be a function of this species taking advantage of human activities and habitation (i.e. food resources or shelter) that result in “dissociated” adrenocortical responses from surrounding habitats. Therefore, it seems the pattern of adrenocortical responses exhibited by human commensal species may be independent of habitat conditions.

Eurasian tree sparrows (*P. montanus*) are resident birds throughout much of their broad distribution in different environments of the Eurasian continent. They can breed one or two times per year on the Tibetan Plateau and two or three times in the lowland areas (Fu et al., '98). Similar to their congeners, house sparrows, Eurasian tree sparrows are also commensals of human activities and habitation in the eastern part of their range.

In order to verify the hypothesis of human commensal species showing dissociated stress responses from surrounding habitats, we examined the seasonal changes of adrenocortical responses to acute stress in Eurasian tree sparrows from lowland areas (67–135 m, benign environments), and compared them with the previously published data from highland (more than 3,200 m, harsh environments) populations (Li et al., 2008). Our first objective was to assess the generality of seasonal CORT modulation by studying the baseline and stress-induced CORT levels (maximal CORT, total and corrected integrated CORT levels) in lowland Eurasian tree sparrows in different life history stages. Our second objective was to compare both the baseline and the stress-induced CORT levels in lowland populations with those at highland during each life history stage, respectively. We predicted that sparrows from both lowland and highland areas would show similar patterns of seasonal modulation in CORT responses. We also predicted there are no significant differences in baseline CORT and stress-induced CORT levels between highland and lowland sparrows during each life history stage, which differ from nonhuman commensal species from harsh and benign environments.

MATERIALS AND METHODS

Species, Study Areas

Free-living Eurasian tree sparrows were captured opportunistically by Japanese mist nets between January 11 and September 23, 2009, at Qianjin village (38°07.278'N 114°30.461'E, elevation: 67 m), Nanshijiazhuang village (38°04.532'N 114°35.092'E, elevation: 135 m), and Luancheng county (37°57.818'N 114°36.837'E, elevation: 56 m). The samples were collected in the early breeding stage (between March 18 and April 13; $N = 12$; six males and six females), the late breeding stage (second nestling stage; between July 25 and August 3; $N = 13$; seven males and six females), the prebasic molt stage (between

September 18 and 23; males and females: $N = 7$), and the wintering stage (between January 14 and February 6; males and females: $N = 8$; we did not take the sex differences into account during these two stages because no significant sex differences were found in avian species studied during the prebasic molt and the wintering stages; see Pravosudov et al., 2004; Romero et al., 2006; Li et al., 2008), respectively.

Capture Stress Protocol and Sampling

Both the lowland and highland populations of Eurasian tree sparrow were sampled using an identical capture stress protocol (Wingfield et al., '92; Li et al., 2008). Immediately after capture, each bird was removed from the net as quickly as possible and approximately 30 μL of blood collected within 2–3 min as baseline CORT level. Then birds were placed in opaque cloth bags and subsequent samples were collected at 10, 30, and 60 min 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 blood vessel with a 26-G needle (Becton, Dickinson and Company, NJ; product no. 305111) and collecting it in heparinized microhematocrit capillary tubes (VWR international, NJ). Blood samples were stored on ice until centrifuged ($855 \times g$ for 10 min) within 3–4 hr. Plasma was collected and stored at -20°C and transported to the Hebei Normal University for the assays 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 Farmer, '78; Biebach et al., '86). Individuals were measured for width and height of cloacal protuberance, and also examined for presence or absence of brood patch and stage of molt. On completion of sampling, all birds were marked for identification with a metal band and released.

Sex identification for Eurasian tree sparrows was determined by the presence of a brood patch in the late breeding and prebasic molt stages. In the early breeding stage, we determined the sex by polymerase chain reaction amplification of the CHD1Z/W gene with the primers 0057F and 002R, following the procedures of Round et al. (2007), after extracting the DNA from the red blood cells.

Enzyme Immunoassay

Plasma CORT levels were detected using Enzyme Immunoassay (EIA) Kits (Cat No. 901-097, Assay Designs), following the methods of Wada et al. (2007). The optimized plasma dilution for Eurasian tree sparrow was 1:42 plasma with 2% steroid displacement buffer. All samples were randomly assigned to one of eight assays. And all were measured in duplicate. The intra- and interassay variations were 4.1 and 8.1%, respectively. Average detectability of assays was 0.86 ng/mL.

Although the previous study used Radioimmunoassay (RIA) methods to determine CORT levels while this study used EIA, both methods gave statistically indistinguishable outcomes when

evaluating the same standard (10 ng/mL; EIA: 10.01 ± 0.54 ng/mL, $N = 8$, RIA: 10.75 ± 0.74 ng/mL, $N = 8$; the data are presented as mean \pm SE). Therefore, it was feasible to compare the CORT data between lowland and highland populations during a specific stage.

Data Analyses

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) 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). The comparisons of body mass, baseline, maximal, total and corrected integrated CORT levels were made by two-way ANOVA with sex and breeding stages as independent variables, by one-way ANOVA (among different life history stages) or independent t -test (between the lowland and the highland populations).

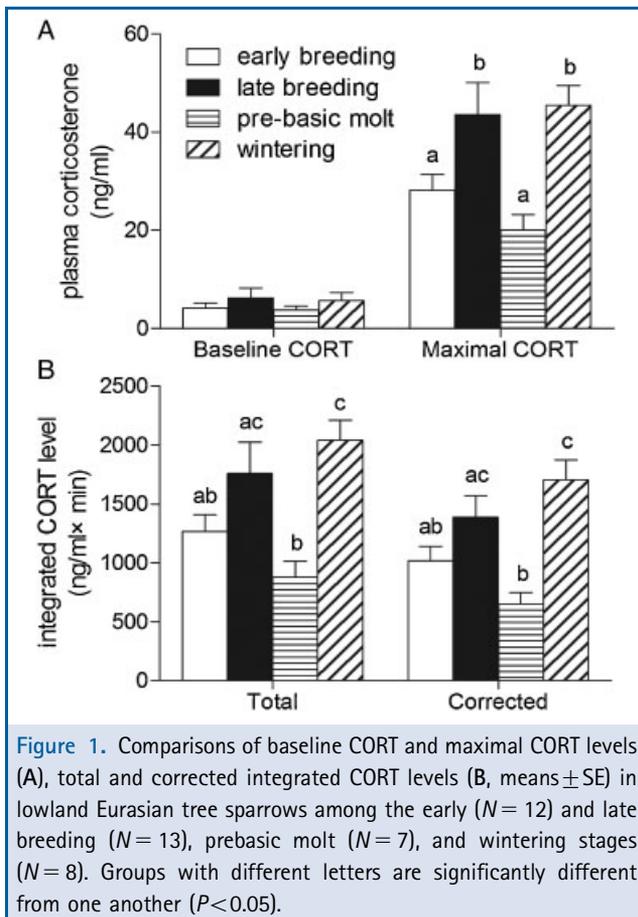
The changes of stress plasma CORT concentrations were analyzed by using repeated measures ANOVA, with time as a repeated factor and with life history stage as between-subjects factor. Bonferroni correction was conducted for multiple comparisons. Before statistical analysis, the homogeneity of variances was tested using Levene's test of equality of variances. If variances among data were significantly different, the data were log transformed to reduce heteroscedasticity. All tests were performed using SPSS16.0 software. All data are presented as means \pm SE.

RESULTS

Seasonal Changes of Adrenocortical Responses in Lowland Area

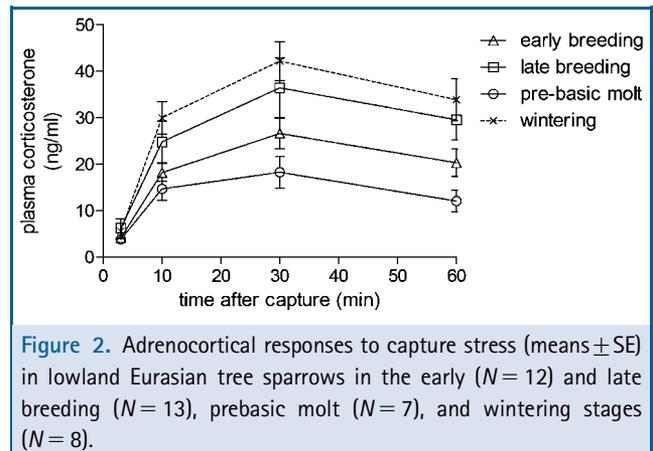
Body Mass and Baseline CORT. Even though there were no differences in baseline CORT levels between the early and late breeding stages ($F_{1,23} = 0.11$, $P = 0.743$), males showed significantly higher levels of baseline CORT than females during the early breeding stage ($F_{1,23} = 0.072$, $P = 0.791$; interaction between sex and stage: $F_{1,23} = 9.719$, $P = 0.005$). There were no differences in body masses between sexes or breeding stages (sex: $F_{1,23} = 0.743$, $P = 0.399$; stage: $F_{1,23} = 0.623$, $P = 0.439$; interaction between sex and stage: $F_{1,23} = 0.889$, $P = 0.357$).

When the data from both sexes were combined (for easier comparison with the preexisting highland dataset, despite the significant differences between the sexes in baseline CORT levels), there were no significant differences in body masses ($F_{3,36} = 0.403$, $P = 0.752$) and baseline CORT levels ($F_{3,36} = 0.492$, $P = 0.69$) among the early and late breeding, the prebasic molt, and the wintering stages in lowland Eurasian tree sparrows (Fig. 1A).



Adrenocortical Response to Capture Stress. Eurasian tree sparrows exhibited significantly increased adrenocortical responses to a standardized handling protocol at different times after capture ($F_{2,2,78.5} = 60.7$, $P < 0.001$). Multiple comparisons revealed that the baseline CORT levels were significantly lower than those CORT levels after 10 min ($P < 0.001$), 30 min ($P < 0.001$), and 60 min ($P < 0.001$), respectively (Fig. 2). The magnitude of this handling stress response showed significant differences among different life history stages ($F_{3,36} = 4.67$, $P = 0.007$; Fig. 2). Multiple comparisons showed that the CORT stress responses during the prebasic molt stage were significantly lower than those in the wintering stage ($P = 0.02$). There was no significant interaction effect between different life history stages and time after capture ($F_{6,5,78.5} = 2.09$, $P = 0.06$; Fig. 2).

Eurasian tree sparrows showed higher maximal CORT levels during the late rather than the early breeding stages ($F_{1,23} = 4.656$, $P = 0.043$), and females had higher maximal CORT levels than males during the late breeding stage ($F_{1,23} = 0.894$, $P = 0.356$; interaction between sex and stage: $F_{1,23} = 4.579$, $P = 0.045$). Even though there were no differences in total integrated CORT levels between sexes ($F_{1,23} = 0.487$,



$P = 0.493$) or breeding stages ($F_{1,23} = 3.297$, $P = 0.084$), females showed higher total integrated CORT levels than males during the late breeding stage (interaction between sex and stage: $F_{1,23} = 6.003$, $P = 0.024$). There were no significant differences in corrected integrated CORT levels between sexes ($F_{1,23} = 0.771$, $P = 0.39$) or breeding stages ($F_{1,23} = 2.96$, $P = 0.101$; interaction between sex and stage: $F_{1,23} = 2.714$, $P = 0.115$).

When the data from both sexes were combined, there were significant differences not only in maximal CORT ($F_{3,36} = 4.595$, $P = 0.008$), but also in total ($F_{3,36} = 4.898$, $P = 0.006$) and corrected integrated CORT levels ($F_{3,36} = 6.526$, $P = 0.001$) among different life history stages. Maximal CORT levels remained significantly lower during the early breeding and prebasic molt stages than those in the late breeding (early breeding: $P = 0.047$; prebasic molt: $P = 0.007$) and the wintering stages (early breeding: $P = 0.02$; prebasic molt: $P = 0.004$; Fig. 1A and B). Similarly, total and corrected integrated CORT levels were significantly lower during the early breeding and prebasic molt stages than those in the wintering stage (total integrated: early breeding, $P = 0.02$; prebasic molt, $P = 0.002$; corrected integrated: the early breeding, $P = 0.005$; prebasic molt, $P < 0.001$), and significantly lower during the prebasic molt stage than those in the late breeding stage (total integrated: $P = 0.008$, corrected integrated: $P = 0.004$; Fig. 1).

Comparison of Adrenocortical Responses Between Lowland and Highland Areas

There were no significant differences in baseline CORT ($F_{1,19} = 0.046$, $P = 0.566$), maximal CORT ($F_{1,19} = 0.256$, $P = 0.292$), and total ($F_{1,19} = 0.201$, $P = 0.977$) and corrected integrated CORT levels ($F_{1,19} = 0.266$, $P = 0.788$) between lowland and highland Eurasian tree sparrows during the early breeding stage, whereas highland sparrows were heavier ($F_{1,19} = 15.287$, $P < 0.001$; Fig. 3) and had a greater fat score than the lowland populations ($F_{1,19} = 9.0$, $P < 0.001$).

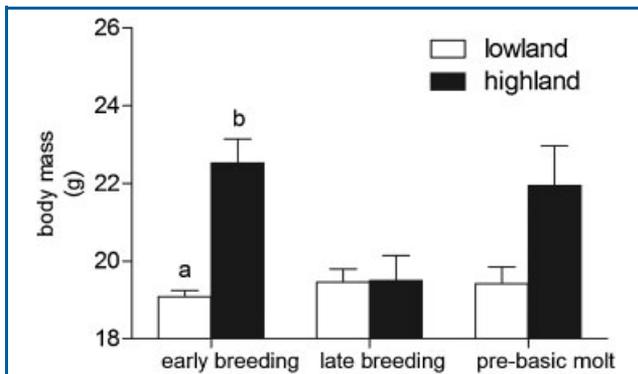


Figure 3. Seasonal comparisons in body masses (means \pm SE) between lowland and highland Eurasian tree sparrows (sample sizes for the early breeding: lowland, $N = 12$, highland $N = 9$; late breeding: lowland $N = 13$, highland $N = 7$; prebasic molt: lowland $N = 7$, highland $N = 10$; the data of highland populations were previously published and reanalyzed with permission from Li et al., 2008 and Elsevier Company, the same below). Groups with different letters are significantly different from one another ($P < 0.05$).

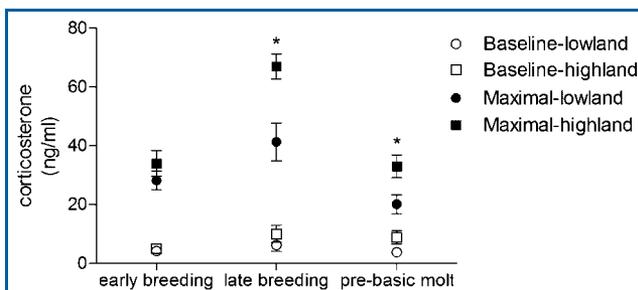


Figure 4. Seasonal comparisons in baseline and maximal CORT levels (means \pm SE) between lowland and highland Eurasian tree sparrows (sample sizes for the early breeding: lowland, $N = 12$, highland $N = 9$; late breeding: lowland $N = 13$, highland $N = 7$; prebasic molt: lowland $N = 7$, highland $N = 10$). The asterisk represents a statistically significant difference ($P < 0.05$).

There were no significant differences in body masses ($F_{1,14} = 0.01$, $P = 0.93$), baseline CORT ($F_{1,18} = 0.611$, $P = 0.29$), and total ($F_{1,18} = 0.303$, $P = 0.58$) and corrected integrated CORT levels ($F_{1,18} = 1.825$, $P = 0.925$) between lowland and highland sparrows during the late breeding stage, whereas highland sparrows showed significantly higher maximal CORT levels than the lowland populations ($F_{1,18} = 1.457$, $P = 0.02$; Fig. 4).

There were no significant differences in body masses ($F_{1,15} = 1.457$, $P = 0.065$) and baseline CORT ($F_{1,15} = 17.673$, $P = 0.059$) between lowland and highland sparrows during the prebasic molt stage, whereas highland sparrows showed significantly higher levels of maximal CORT ($F_{1,15} = 0.619$, $P = 0.027$) and total ($F_{1,15} = 0.882$, $P = 0.004$) and corrected

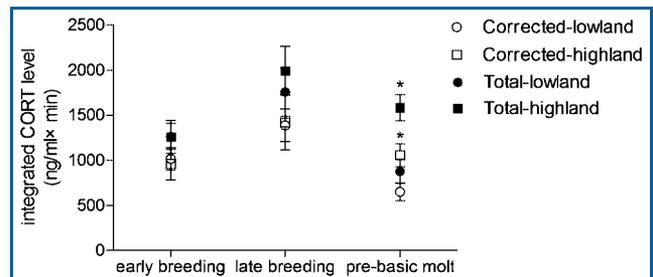


Figure 5. Seasonal comparisons in total and corrected integrated CORT levels (means \pm SE) between lowland and highland Eurasian tree sparrows (sample sizes for the early breeding: lowland, $N = 12$, highland $N = 9$; late breeding: lowland $N = 13$, highland $N = 7$; prebasic molt: lowland $N = 7$, highland $N = 10$). The asterisk represents a statistically significant difference ($P < 0.05$).

integrated CORT levels ($F_{1,15} = 0.626$, $P = 0.034$) than lowland populations (Figs. 4 and 5).

DISCUSSION

In this study, the baseline CORT levels of lowland Eurasian tree sparrows did not differ among life history stages, and the stress-induced CORT levels (maximal CORT and total and corrected integrated CORT levels) were lower during the early breeding and prebasic molt stages than those in the late breeding and wintering stages. As expected, the seasonal modulation patterns of baseline and stress-induced CORT levels in lowland Eurasian tree sparrows are similar to the highland populations. However, the highland sparrows had significantly higher maximal CORT levels during the late breeding stage and higher stress-induced CORT levels during the prebasic molt stage.

Seasonal Changes of Adrenocortical Responses in Lowland Eurasian Tree Sparrows

Many passerine species show a seasonal peak of baseline CORT levels during the early breeding stage then decreasing CORT as breeding commences to a nadir during the prebasic molt stage (reviewed by Romero, 2002; see also Holberton and Wingfield, 2003; Wingfield and Sapolsky, 2003). However, in this study, the baseline CORT levels in lowland sparrows did not show seasonal pattern (except males had higher baseline CORT than females during the early breeding). Our results are consistent with previous studies showing no seasonal changes in baseline CORT levels of the Tibetan Plateau populations among the late breeding and the prebasic molt stages (Richardson et al., 2003; Li et al., 2008), as well as no changes in the baseline CORT levels of house sparrows throughout the year (both total and free CORT, Romero et al., 2006; free CORT, Breuner and Orchinik, 2001). Baseline CORT levels may function as a physiological index of relative condition or health of individuals, which can mediate a trade-off in self-maintenance vs. reproduction and vary with the energetic

condition (Marra and Holberton, '98; Walker et al., 2005; Wikelski and Cooke, 2006; Bonier et al., 2009). Interestingly, males had higher baseline CORT levels than females during early breeding stage in the lowland areas, whereas there were no such differences in highland populations (Li et al., 2008). The degree of parental expenditure of male birds can vary with altitude. For example, males from highland areas (harsher, less predictable conditions) may increase the investment of time and energy in parental care than those from lowland areas (Badyaev and Ghalambor, 2001; Johnson et al., 2007). Whether this difference reflects the highland sparrows having increased male parental care remain to be determined.

Supporting previous findings in highland populations (Richardson et al., 2003; Li et al., 2008), lowland Eurasian tree sparrows in our study showed significantly lower stress-induced CORT levels during the prebasic molt than those in the late breeding stage (Fig. 1A and B). Our results are also consistent with another human commensal species, house sparrow (Hegner and Wingfield, '90; Breuner and Orchinik, 2001; Romero et al., 2006) and most other passerine species (Reviewed by Romero, 2002; but see Romero and Wingfield, 2001). The reduced stress-induced CORT level during the prebasic molt stage is necessary for producing high-quality feathers by inhibiting the protein deposition and preventing the protein catabolic activity of CORT (Romero et al., 2005).

Moreover, we found that the stress-induced CORT levels during the wintering stage were significantly higher than those in the early breeding and prebasic molt stages, which is consistent with previous reports in house sparrow (Romero et al., 2006). This seasonal pattern is similar to those species inhabiting harsh (Wingfield et al., '94) and desert environments (breeding season vs. nonbreeding season, Wingfield et al., '92). The stress-induced CORT levels are secreted in response to life-threatening and unpredictable perturbations, which can contribute to optimizing their survival by producing emergency strategies, behaviorally and physiologically (Wingfield et al., '97; Landys et al., 2006).

Comparison Between Lowland and Highland Areas

In some avian species, although the energetic demands were presumably higher in high-latitude populations than those of low latitude, the baseline CORT levels did not necessarily vary with latitude (Silverin and Wingfield, '98; Breuner et al., 2003; Wilson and Holberton, 2004), but did in White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) and American tree sparrows (*Spizella arborea*; Holberton and Wingfield, 2003). Similarly, our results showed that highland Eurasian tree sparrows were in better energetic condition than lowland populations during the early breeding stage (with heavier body mass and greater fat score), but the baseline CORT levels did not change with altitude among life history stages. Baseline CORT levels may help individuals to sustain normal activity patterns by regulation of feeding, locomotor activity, energy metabolism, and immune

system to cope with daily or seasonal demands of the predictable life history cycle (Padgett and Glaser, 2003; Landys et al., 2006). In this study, the better energetic condition in highland populations during the early breeding stage may be an ecological strategy to prepare for the periods of stringency in an unpredictable environment and less predictability in food resources.

In addition, highland sparrows showed significantly higher maximal CORT levels during the late breeding than lowland populations (Figs. 4 and 5). Generally, maximal CORT may reflect the instantaneous reactivity of individuals in response to handling stress, whereas total and corrected CORT levels reflect the durative reactivity (Breuner et al., 2008). Accordingly, we can predict highland sparrows express more sensitive and temporal reactivity (rapid increase of CORT and then restore to normal) than lowland populations. Whether the sensitive and temporal reactivity can promote the survival (through self-maintenance behaviors) of highland sparrows during late breeding (after juveniles left the nest) remains to be determined.

In avian species, the modulation of adrenocortical responses to acute stress can vary with geographic differences during breeding stage. Compared with conspecifics breeding in benign environments, those breeding in harsh environments showed lower adrenocortical responses, possibly a mechanism to maximize their reproductive success by avoiding the potentially disruptive effects of severe weather (Wingfield et al., '92, '94; Silverin et al., '97; Silverin and Wingfield, '98; Wingfield and Hunt, 2002; Breuner et al., 2003; Wilson and Holberton, 2004). However, both lowland and highland Eurasian tree sparrows showed similar patterns in modulating adrenocortical responses during the early and late breeding stages (except for maximal CORT level during the late breeding stage). Our results are consistent with house sparrows showing nearly equivalent CORT responses in both benign and harsh environments (Romero et al., 2006). The potential reason may lie in both the Eurasian tree sparrows and house sparrows being human commensal species, and they can take advantage of human buildings and food resources, and thus are shielded from the climatic and other conditions of the surrounding habitats (Romero et al., 2006; Li et al., 2008; Wingfield et al., 2011). Therefore, it seems true commensals of humans (i.e. species that is generally only found in the presence of human activities, such as Eurasian tree sparrows and house sparrows) show "dissociated" patterns of adrenocortical responses to stress from surrounding habitats, whereas urbanized species (i.e. that have colonized human habitations but also have populations completely independent of humans, such as Florida scrub jays *Aphelocoma coerulescens*, European blackbirds *Turdus merula* and white-crowned sparrows) may even show opposite responses (Schoech et al., 2004; Partecke et al., 2006; Bonier et al., 2007a,b).

Interestingly, the highland Eurasian tree sparrows showed stronger adrenocortical responses during the prebasic molt stage than lowland ones. Previous studies demonstrated that in

white-rumped snowfinches (*Onychostruthus taczanowskii*), Rufous-necked snowfinches (*Pygilauda ruficollis*), and Eurasian tree sparrows on the Tibetan Plateau showed the same magnitude increases of CORT responses in the prebasic molt stage as the early breeding (Richardson et al., 2003; Li et al., 2008). Unlike the arctic breeding birds, the increases in CORT responses are very robust during the early breeding stage and very small or absent during the prebasic molt stage (Astheimer et al., '94; Romero et al., '97, '98; Holberton and Wingfield, 2003). This small increase in adrenocortical responses during the prebasic molt stage may contribute to maximizing their survival. Whether it is a unique physiological and ecological adaptation strategy for adapting to harsh environments remains to be further determined.

Taken together, our results suggest that (1) similar to highland Eurasian tree sparrows, lowland populations show no differences in baseline CORT levels among life history stages, whereas the stress-induced CORT levels are lower during the early breeding and prebasic molt stages than those in the late breeding and wintering stages. Such similar pattern may correlate with human commensal species showing "dissociated" patterns of adrenocortical responses to stress from surrounding habitats by taking advantage of human buildings and food resources; (2) highland Eurasian tree sparrows show stronger adrenocortical responses during the prebasic molt stage than those of the lowland populations. It seems the Eurasian tree sparrows from both harsh and benign environments have similar patterns of adrenocortical responses in the breeding stage (except for maximal CORT during the late breeding), whereas they are different in the prebasic molt stage. In highland birds, the increased maximal CORT levels during the late breeding and the small but not robust increases in adrenocortical responses during the prebasic molt stage are interesting, but remain to be further explored.

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