What drives variation in the corticosterone stress response between subspecies? A common garden experiment of swamp sparrows (*Melospiza georgiana*)

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**Abstract**

Although differences in the corticosterone stress response have frequently been reported between populations or closely related subspecies, their origin remains unclear. These differences may appear because individuals adjust their corticosterone stress response to the environmental conditions they are experiencing. However, they may also result from selection that has favoured individuals with specific corticosterone stress response or from environmental factors that have affected the development of the corticosterone stress response during early life. We investigated these hypotheses by studying the corticosterone stress response of two closely related subspecies of swamp sparrows (*Melospiza sp*). We showed for the first time that two closely related subspecies can differ in their corticosterone stress response when raised at the laboratory and held in similar conditions for a year. Thus, we demonstrated that selection, developmental processes or a conjunction of both of these processes can account for variation in the stress response between closely related subspecies.

corticosteroid hormones, i.e. corticosterone or cortisol (the glucocorticosteroid stress response, Wingfield, 1994, 2003; Wingfield *et al.*, 1998). This endocrine response promotes the occurrence of behavioural and physiological forms that enhance the immediate survival, but also redirects resources away from growth, reproduction, growth and moultng processes (Sapolsky *et al.*, 2000; Wingfield & Sapolsky, 2003; Romero *et al.*, 2005; Wada, 2008; Wada & Breuner, 2008; Müller *et al.*, 2009). This stress response is particularly relevant when studying life-history decisions because it is now well referenced that individuals can modulate their glucocorticoid stress response according to the fitness costs and benefits that the activation of an emergency life-history stage provides (Hoberton *et al.*, 1996; Wingfield & Sapolsky, 2003; Heidinger *et al.*, 2006; Lendvai *et al.*, 2007; Angelier *et al.*, 2009a). For instance, the stress response is usually attenuated during the reproduction, the moult or the chick’s growth, and this has been interpreted as a way for individuals to reduce the important negative effects of elevated corticosterone levels on chick’s development, feather growth and reproduction (Sims & Hoberton,
Interestingly, differences in the corticosterone stress response have frequently been reported between bird populations or closely related bird subspecies (Astheimer et al., 1994; Marra et al., 1995; Wingfield et al., 1995, 2008b; Holberton & Able, 2000; Breuner et al., 2003; Wilson & Holberton, 2004; Lindstrom et al., 2005; Wada et al., 2006; Müller et al., 2007; Horton & Holberton, 2010). For instance, Silverin et al. (1997) found that the corticosterone stress response varies between two populations of willow warblers (Phylloscopus trochilus) that cope with contrasting environmental situations. Similarly, tropical, temperate and arctic subspecies of the well-studied white-crowned sparrows (Zonotrichia sp.) show contrasted corticosterone stress responses, and this can be related to different environmental contexts (Astheimer et al., 1994; Breuner et al., 2003; Wada et al., 2006; Wingfield et al., 2008b). Although environmental conditions and life-history strategies can be related to these differences in the corticosterone stress response between populations and subspecies (Wingfield et al., 1995; Breuner et al., 2003; Holberton & Wingfield, 2003; Clinchy et al., 2004; Wilson & Holberton, 2004; Wada et al., 2006; Müller et al., 2007; Almasi et al., 2009; Bokony et al., 2009; Hau et al., 2010; Horton & Holberton, 2010), the origin of these differences in the corticosterone stress response between populations and subspecies remains unclear. The corticosterone stress response may differ between subspecies or populations because they live in different areas and, thus, adjust their corticosterone stress response to the contrasted environmental conditions that they experience. On the other hand, populations and subspecies may differ in the corticosterone stress response because of selection pressure that has favored individuals with specific corticosterone stress response (Satterlee & Johnson, 1988; Evans et al., 2006; Wada et al., 2009; Almasi et al., 2010) or because of environmental factors that have affected the development of the HPA axis during early life and, thus, the corticosterone stress response that adults can mount (Love & Williams, 2008a,b; Wada et al., 2009). Unfortunately, it is very challenging to disentangle these hypotheses because populations are sampled in contrasting environments and, at the same time, may have been confronted to different selection pressure and developmental conditions (Müller et al., 2007).

Here, we studied two closely related subspecies of passerine (swamp sparrows, Melospiza georgiana) that live in contrasted environments (Olsen et al., 2008, 2010; Ballentine & Greenberg, 2010) and we experimentally tested whether the intensity of their corticosterone stress response differs when they are held in similar environmental conditions for an extended period of time. Specifically, we experimentally controlled for the influence of environment by conducting a common garden experiment: chicks from these two subspecies were reared in captivity and maintained under similar environmental conditions for more than 1 year (Ballentine & Greenberg, 2010). We then asked whether the intensity of the corticosterone stress response differs despite these two groups being reared in the same environmental condition. By doing so, we are able to better understand what drives variation in the corticosterone stress response and its phenotypic plasticity, between closely related groups such as subspecies: (1) if the corticosterone stress response of individuals of the two subspecies of swamp sparrows has been shaped by selection and/or developmental processes, we predict that swamp sparrows subspecies will differ in their corticosterone stress response and, this, despite the similar environmental conditions of this ‘common garden’ experiment; (2) if individuals of these two subspecies adjust their corticosterone stress response to the environmental conditions that they are experiencing, we predict that the two subspecies of swamp sparrows will show a similar corticosterone stress response because of the similar environmental conditions of the common garden experiment.

Methods

Study populations and subspecies

Our study focuses on how the corticosterone stress response differs between two closely related subspecies of swamp sparrows that live in two contrasted environments (Olsen et al., 2008, 2010; Ballentine & Greenberg, 2010). Our study population of Melospiza g. nigrescens birds is found in tidal areas within state game lands near Woodland Beach, Delaware, USA (75.6°W, 39.4°N), whereas the study population of Melospiza g. georgiana birds is located 319 km inland in cranberry fens on the Allegheny Plateau in Garrett County, Maryland, USA (79.3°W, 39.6°N). Although these two sites are located at the same latitude, they differ significantly in altitude (800 m), predation risk, habitat structure and climate (Olsen, 2007) – four factors that are known to affect the corticosterone stress response (habitat: Marra & Holberton, 1998; Busch & Hayward, 2009; altitude: Wingfield et al., 2008b; predation risk: Clinchy et al., 2004; Berger et al., 2007; Thiel et al., 2008; climate: Romero et al., 2000; Busch et al., 2010; Hau et al., 2010). Indeed, these two subspecies of swamp sparrows have diverged very recently as suggested by the absence of consistent differences in their mitochondrial DNA markers (Greenberg et al., 1998) and are distinguished by only a few morphological and vocal differences (Greenberg & Droge, 1990; Ballentine, 2006; Liu et al., 2008; Ballentine & Greenberg, 2010; Greenberg & Olsen, 2010). In addition, these two subspecies differ in life-history strategy (i.e. another component strongly related to the corticosterone stress response; Bokony et al., 2009; Hau et al., 2010), with Melospiza g. nigrescens birds investing less into their current reproduction and behaving more as
prudent birds than Melospiza g. georgiana birds do (Olsen et al., 2008, 2010).

The common garden experiment

We located the nests of breeding swamp sparrows in Woodland Beach Wildlife Area, Delaware (Melospiza g. nigrescens) and The Glades, Garrett County Maryland (Melospiza g. georgiana). Nests were monitored until hatching, and we collected nestlings when they reached 4 days of age. We collected 16 nestlings of Melospiza g. georgiana (five nests) from Maryland and 16 nestlings of Melospiza g. nigrescens (six nests) from Delaware. We transported nestlings to indoor animal care facilities at the Smithsonian National Zoological Park, Washington, DC where we hand reared them under identical conditions on 12D:12L photoperiod. Nestlings fledged at approximately 10 days and were transferred into group cages. Once nestlings reached independence at approximately 18 days, they were transferred into individual cages (18”L x 9” D x 10½” H) where they remained into adulthood on natural photoperiod cycles. All birds were sexed by genetic assignment (Griffiths et al., 1998).

Nestling diet was a mixture of raw lean ground beef, whole grain baby cereal, raw wheat germ, hard boiled egg, carrot, calcium supplement, iron supplement, multi-vitamin supplement and powdered milk. Nestlings were hand-fed once every half hour until day 10, then once every hour. At day 18, fresh food (see below) was introduced ad libitum and the hand feeding frequency was reduced to once every 3 h. At day 24, hand feeding ceased as they birds were eating on their own. Fresh food comprising this rearing diet was provided ad libitum along with an adult diet (see below) for 1 week and then the fresh food rearing diet was reduced gradually over the course of the 3 weeks until birds were on an adult fresh food diet by approximately 60 days of age (Day 60). Adult fresh food diet was a combination of soaked seed, fresh peas, tofu and egg food. This diet provided birds with a variety of items to choose from while they were becoming independent. Adult diet was provided ad libitum and comprised dry seed mixture, 6–8 mealworms every other day and egg food, with shell and multi-vitamin once per week. The birds were provided a commercially available grit that contained a calcium supplement.

Sampling period

In this study, swamp sparrows were sampled after the end of the breeding cycle during the moult period (13–27 August 2008). They were not sampled during the breeding period because it was difficult to accurately determine breeding and reproductive status, as such, in captivity: although all sparrows were kept in individual cages and were not allowed to breed in our common garden experiment, they exhibited signs of breeding condition and showed high inter-individual and inter-sexual variations in reproductive behaviour, such as singing rates, courting behaviour in males and copulation solicitations in females (Ballentine & Greenberg, 2010). Because the corticosterone stress response is known to be modulated according to the fitness value of the current reproduction (Holberton & Wingfield, 2003; Wingfield & Sapolsky, 2003; Lendvai et al., 2007), interpreting variations in the corticosterone stress response between individuals of unknown reproductive status would have been problematic. To avoid this potential source of variation, swamp sparrows were sampled after their first breeding cycle, during moult.

Body condition and corticosterone stress response

We measured wing length (unflattened wing chord) and tail length to the nearest 0.1 mm. These two measurements were highly correlated ($n = 32, r = 0.832, P < 0.001$), and therefore, we calculated a measure of body size for each from a factor analysis. Factors were extracted by a principal components analysis performed on the two measurement variables (tail length, wing length). The first axis accounted for 90.54% of variance, and the resulting factor for each individual (PC1) was therefore assumed to represent overall body size. Because these two measurements are affected by the moult progress, we measured wing and tail length before the onset of moult to be sure to obtain a relevant measure of body size. We then calculated our residual indices of body condition by using the residuals from a regression of body mass against our body size factor ($n = 32, r = 0.478, P = 0.006$).

All birds were bled according to the standardized technique described by Wingfield (1994). Immediately after entering the chamber (i.e. the room where all the cages were located), an initial, hereafter referred to as ‘baseline’, small blood sample (80 µL) was collected from the alar vein with a 27-gauge needle. A second small sample (80 µL) was taken 30 min after the time the chamber was entered. Sparrows were kept in individual opaque cloth bags between the initial and second blood samples. All the initial samples were collected within 3 min of entering the chamber, to ensure that plasma CORT levels were not elevated because of the stress of disturbance and capture (Romero & Reed, 2005; Müller et al., 2007; Angelier et al., 2010). Moreover, birds were sampled either at 10:00 or 15:00 and within 2 weeks to avoid the possibility of baseline or stress-induced corticosterone levels being affected by diurnal and seasonal rhythm in corticosterone secretion. We did not find any difference in either baseline or stress-induced corticosterone levels between 10:00 and 15:00 for each species (ANOVA, $P > 0.500$ for all tests), and baseline and stress-induced corticosterone levels did not vary with the date of sampling (linear regressions, $P > 0.300$ for all tests). This demonstrates that moult progress did not affect the stress response in our study as previously demonstrated.
in birds (Butler & Romero, 2009). Therefore, moul
top progress is unlikely to account for difference in the stress
response between subspecies. In addition, we did not find any
effect of the nest of origin on baseline or stress-
induced corticosterone levels for Melospiza g. nigrescens
(ANOVA, P > 0.500 for all tests). Unfortunately, we could
not check this for Melospiza g. georgiana because the
chicks of this subspecies simultaneously fledged before
they could be individually marked and it was impossible
to know the nest of origin for these chicks (all the nests
had been transferred from the wild into a single cage).
However, the results from Melospiza g. nigrescens suggest
that the nest of origin is very unlikely to affect cortico-
sterone levels measured during the moul. This project
was approved by the Smithsonian Institution Animal
Care and Use Committee (#2007-03).

Corticosterone assay
After collection, blood samples were immediately cen-
trifuged for 10 min (speed: 10 000 rpm, 13 000 g). Plasma
was collected and then stored at −20 °C until analysed
for corticosterone concentration by radioimmunoassay
at the laboratory of Avian Biology at the University of
Maine, USA, as previously described (Wingfield et al.,
1992). Briefly, samples were allowed to equilibrate
overnight with 2000 cpm of corticosterone. Four millili-
litres of distilled dichloromethane was added to each
sample to extract corticosterone. The steroid containing
organic phase was then aspirated and the solvent
evaporated under nitrogen in a 40 °C water bath. The
extracts were reconstituted in 550 mL of phosphate-
buffered saline. Samples were then assayed in duplicate.
A series of pairs of replicates containing decreasing
concentrations of unlabelled corticosterone (Sigma,
St. Louis, MO, USA) and 100 μL of antibody and 100 μL of radiolabelled corticosterone was set up to
create a standard curve from which all sample values
were determined (see Cash & Holberton, 1999 for further
details). The sensitivity of the assay, based on the
standard curve, was 7.8 pg mL⁻¹. No samples fell below
the detection limit of the assay. All samples were run
within a single assay, eliminating inter-assay variation.
The within-assay variation coefficient of variation was
15%, based on a commercial standard.

Statistics
All analyses were performed with SAS statistical software
(SAS Institute Inc. v 9.1, Chicago, IL, USA). We used the
information-theoretic approach to identify suitable mod-
els for predicting body condition and corticosterone levels
in the two subspecies of swamp sparrows (Burnham &
Anderson, 2002). Candidate models were constructed
a priori with explanatory variables hypothesized to influence
either body condition or corticosterone levels (e.g.
sex, subspecies, body condition). For all variables tested,

normality assumptions were checked by plotting the
residuals against the predicted value and by running the
Shapiro–Wilk test for normality. There were no indica-
tions that normality assumptions were violated. There-
fore, we used generalized linear models with normal
errors and an identity link function to explain the effect of
our variables of interest on body condition and cortico-
sterone levels. Model fitting was performed by maximum
likelihood with the PROC GENMOD procedure in SAS;
we used maximum likelihood techniques and Akaike’s
Information Criterion (AICc) statistics corrected for small
sample size to compare the extent to which our data fitted
each model (Burnham & Anderson, 2002). We calculated
this second-order AICc using this formula:

\[
\text{AICc} = -2 \log(\text{Likelihood}) + 2K + 2(K + 1)\frac{K}{N - K - 1},
\]

where \(N\) is the sample size and \(K\) the number of
parameters.

Values for differences in AICc values (ΔAICc) were
computed by subtracting the minimum AICc from all
candidate model AICc. Differences between AICc values
different models can be used to determine which
model provides the most adequate description of the data
on the basis of the fewest model parameters. The model
with the lowest AICc is considered the best description of
the relationship. ΔAICc values > 2 are a good indicator
that the model with the lowest AICc is preferable. ΔAICc
values < 2 indicate that models are fairly similar in their
ability to describe the data, and the model including the
fewest parameters is usually preferable.

First, we tested whether body condition was affected by
sex, subspecies and their interaction (Table 1, Model 1).
Second, we tested whether baseline corticosterone levels
were affected by sex, subspecies, body condition and
their interactions (Table 1, Model 2). Finally, we tested
whether stress-induced corticosterone levels were
affected by sex, subspecies, body condition and their
interactions (Table 1, Model 3). In this study, the stress
response was expressed as stress-induced corticosterone
levels (measured after 30 min of restraint). We also
expressed the corticosterone stress response as the rate of
increase from baseline to stress-induced corticosterone
levels per minute (ng mL⁻¹ min⁻¹). This latter measure
was highly correlated with stress-induced corticosterone
levels in swamp sparrows (\(n = 32, r = 0.979, P < 0.0001\)),
and our statistical analyses gave nearly identical results
for these two measures. Therefore, we report only the
results for stress-induced corticosterone levels to facilitate
the comparison with other published results.

Results
Body condition
We found by using our maximum likelihood approach that
no explanatory variable explained body condition.
According to the parsimony principle, the reduced model – which contained only the intercept term – was preferred over the more complex models (Table 2, Model 1). Therefore, sex and subspecies were not linked to body condition in our captive swamp sparrows (Fig. 1a). Overall, all individuals from both sexes and both subspecies were in very good body condition and had very large fat reserves.

**Baseline corticosterone levels**

The model selection demonstrated that baseline corticosterone levels were not influenced by any independent variable or interaction. According to the parsimony principle, the reduced model – which contained only the intercept term – was preferred over the more complex models (Table 2, Model 2). Specifically, baseline corticosterone levels did not differ between subspecies and sexes (Fig. 1b). Moreover, baseline corticosterone levels were not correlated with body condition. Overall, baseline corticosterone levels were very low for all individuals regardless of subspecies or sex (Mean ± SE: 1.92 ± 0.33, Fig. 1).

**Stress-induced corticosterone levels**

In all individuals, stress-induced corticosterone levels increased in response to the standardized stress protocol (Fig. 2). The model selection demonstrated that stress-induced corticosterone levels were not influenced by body condition. However, the sex and species factors and their interaction were included in all the best four models (Table 2, Model 3). The model selection demonstrated therefore that there was an effect of subspecies on stress-induced corticosterone levels and that, in addition, this effect was only apparent for males. Specifically, stress-induced corticosterone levels were much higher in male *M. g. nigrescens* than in the three other groups (parameter estimates: male *M. g. georgiana*, $\chi^2 = 24.53$, $P < 0.001$; female *M. g. nigrescens*, $\chi^2 = 9.05$, $P = 0.003$; or female *M. g. georgiana*, $\chi^2 = 9.12$, $P = 0.002$; Fig. 1c). On the other hand, stress-induced corticosterone levels did not

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Table 1 Biological assumptions tested and associated generalized linear models. Model selection was performed by using maximum likelihood techniques (Burnham & Anderson, 2002) and Akaike’s information criterion corrected for small samples (AICc).

<table>
<thead>
<tr>
<th>Model</th>
<th>Biological assumption</th>
<th>Dependent variables</th>
<th>Independent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of sex and subspecies on body condition</td>
<td>Body condition</td>
<td>Species, sex and interactions</td>
</tr>
<tr>
<td>2</td>
<td>Effect of sex, subspecies and condition on baseline corticosterone levels</td>
<td>Baseline corticosterone levels</td>
<td>Species, sex, body condition and interactions</td>
</tr>
<tr>
<td>3</td>
<td>Effect of sex, subspecies and condition on the corticosterone stress response</td>
<td>Stress-induced corticosterone levels</td>
<td>Species, sex, body condition and interactions</td>
</tr>
</tbody>
</table>

Table 2 Model selection using Akaike’s Information Criterion (AICc) to explain body condition, baseline and stress-induced corticosterone levels of two subspecies of swamp sparrows held in a common garden experiment. Generalized linear models with normal distribution and identity link were used to test for variable effect. $K$ indicates the number of parameters. Sex and species indicate, respectively, sex of the individual (males vs. females) and subspecies of the individual (*Melospiza g. georgiana* vs. *M. g. nigrescens*). Condition indicates the body condition of the individual, which is calculated as the residuals from a regression of body mass against body size. The four most parsimonious models and the model without any explanatory variable (the ‘Intercept model’) are represented and are ranked according to their AICc. For each model selection, the selected best model is in bold.

<table>
<thead>
<tr>
<th>Model</th>
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<th>Log likelihood</th>
<th>AICc</th>
<th>$\Delta$AICc</th>
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</table>
differ between female *M. g. nigrescens*, female *M. g. georgiana* and male *M. g. georgiana* (parameter estimates: $P > 0.200$ for all $P$-value; Fig. 1c). Overall, stress-induced corticosterone levels were very low except for *M. g. nigrescens* males that have moderately elevated levels (Fig. 1c).

Discussion

In this study, we showed for the first time that two closely related subspecies of birds can differ in their corticosterone stress response when raised at the laboratory and held in a common garden experiment. We demonstrated that variation in the stress response between populations and subspecies probably does not only result from the contrasted environmental conditions in which they are experiencing, but also from selection, developmental processes or an interaction of both of these processes.

What factors drive variations in the corticosterone stress response between subspecies?

We found that males from the two closely related subspecies used in this study differed in their corticosterone stress response despite the similar environmental conditions of our common garden experiment. Although such a difference between populations and closely related subspecies has previously been reported in free-living birds (Wingfield et al., 1995, 2008b; Silverin et al., 1997; Breuner et al., 2003; Wada et al., 2006; Bokony et al., 2009; Hau et al., 2010; Horton & Holberton, 2010), its origin remained poorly understood. Of course, environmental conditions can, by themselves, affect the magnitude of the corticosterone stress response in vertebrates.
and can, under circumstances, explain these differences between populations or subspecies (Marra & Holberton, 1998; Kitaysky et al., 1999; Romero, 2002; Almasi et al., 2009; Bokony et al., 2009; Hau et al., 2010). However, at least for the species used in this study, our common garden experiment demonstrates that such inter-population variation in the corticosterone stress response does not only result from the contrasted environmental conditions that are experienced by the individuals at the time of sampling.

Because these two swamp sparrow subspecies differ in their corticosterone stress response despite a very recent phylogenetic divergence (Greenberg et al., 1998), our results suggest that evolutionary processes may act quickly to shape the corticosterone response to stress in vertebrates. Indeed, the corticosterone stress response is probably subject to strong selection in vertebrates for two main reasons. First, this stress response is genetically determined and heritable (Satterlee & Johnson, 1988; Evans et al., 2006; Almasi et al., 2010). Second, the ability of individuals to mount a corticosterone stress response has important fitness consequences (Breuner et al., 2008; Angelier et al., 2009b; Romero & Wikelski, 2010) because it can provide benefits by helping individuals surviving to a stressor but can also induce costs in term of, for instance, energy use, reproductive success and moult quality (Wingfield et al., 1998; Sapolsky et al., 2000; Wingfield & Sapolsky, 2003; Romero et al., 2005; Wada & Breuner, 2008). Therefore, individuals are probably selected for mounting a stress response, which is well-adjusted to their environment and, which optimizes their fitness. Although we controlled partially for developmental factors by keeping the birds in similar conditions from the age of 4 days old until the period of sampling, we cannot exclude that environmental conditions may have acted on the physiological development of individuals (Badayev & Uller, 2009; Mousseau et al., 2009; Wada et al., 2009). Indeed, eggs were conceived and incubated in two different environments by different parents, and the nestlings of these two closely related subspecies of swamp sparrows did not cope with the same environment during their first 4 days of life. Supporting this possibility, it has been experimentally shown that the corticosterone stress response of birds can be affected by the prenatal and the post-natal conditions they have experienced (starlings, *Sturnus vulgaris*: Love et al., 2008a; Cyr & Romero, 2007; zebra finches, *Taeniopygia guttata*, Wada et al., 2009; but see Lendvai et al., 2009, house sparrows, *Passer domesticus*). Consequently, our common garden experiment demonstrates that selection and/or developmental processes can explain why subspecies and populations with contrasted environmental constraints and strategies differ in their corticosterone stress response.

In addition, these subspecies might also differ in their corticosterone stress responses because of contrasted habituation to captivity (Cyr & Romero, 2009). Thus, *M. g. nigrescens* males might not habituate as well to repeated stressors as *M. g. georgiana* males. This possibility does not contradict our previous interpretations as it would mean that the ability of swamp sparrows to habituate to captivity and, thus, to stressors was not related to the environmental conditions they had experienced since their arrival at the laboratory, but rather to selection and developmental processes.

Finally, we found that, contrary to males, female *M. g. nigrescens* showed a similar corticosterone stress response than female swamp sparrows *M. g. georgiana*. Such sex difference in the intensity of the corticosterone stress response is not so surprising as it has already been reported in numerous species (Wingfield et al., 1995; O’Reilly & Wingfield, 2001; Romero, 2002; Holberton & Wingfield, 2003; Horton & Holberton, 2010). Our results suggest that selection processes may act differently on males and females of each subspecies to shape their corticosterone stress response (Satterlee & Johnson, 1988; Evans et al., 2006; Almasi et al., 2010). In addition to such selection processes, early developmental conditions may also account for these differences in the stress response because subspecies and sexes may have been confronted to contrasted maternal effects or early post-hatching conditions (i.e. four-first days of life, Badayev & Uller, 2009; Mousseau et al., 2009; Wada et al., 2009). Supporting this possibility, Love & Williams (2008a,b) found that, under some circumstances, parents invest more into nestlings of one sex than another, resulting therefore in contrasted energetic situations and, thus, in different development of the corticosterone stress response between male and female nestlings.

**Why do swamp sparrows differ in their stress response?**

From an ecological point of view, these differences in the stress response of swamp sparrows are intriguing and may originate from contrasted costs and benefits of mounting an important stress response between sexes and subspecies (Wingfield & Sapolsky, 2003; McNamara & Buchanan, 2005).

First, these differences could result from contrasted strategies and personalities between sexes and subspecies. For instance, it is predicted that aggressive birds have a better access to resources (food, shelters, mates, etc.) than shy birds. Therefore, aggressive birds can afford to react strongly to a stressor, and temporarily neglect these resources, because their aggressiveness will allow them to easily recover their resource after the stressful period. In contrast, it is probably costly for a shy bird to mount such an important stress response because this bird may then loose its access to resources to the profit of a more aggressive individual and, thus, will not be able to recover these resources after the stressful period (Wingfield & Sapolsky, 2003). Interestingly and similar to previous studies (Pravosudov et al., 2003; Poisbleau et al., 2006; Almasi et al., 2006; Hau et al., 1999; Romero, 2002; Almasi et al., 2010; Bokony et al., 2009).
our results followed this prediction because M. g. nigrescens males are more territorial and aggressive and showed higher stress responses than M. g. georgiana males or females of both subspecies (Olsen et al., 2010).

Second, these differences may result from different life-history strategies between sexes and subspecies (Bokony et al., 2009; Hau et al., 2010). It is now well known that the stress response is overall attenuated when individuals are engaged in reproductive activities (Wingfield & Sapolsky, 2003). Interestingly, in captive male swamp sparrows, M. g. georgiana have larger cloacal protuberance than M. g. nigrescens during the moult (Ballentine & Greenberg, 2010), suggesting that M. g. georgiana have a higher reproductive effort than M. g. nigrescens, even during the moult. These contrasted life-history strategies may therefore explain why M. g. nigrescens males showed a stronger stress response than M. g. georgiana in our study.

Finally, these differences may result from the costs of mounting a strong stress response: elevated corticosterone levels inhibit feather growth and induce a moult of poor quality, which negatively affects thermoregulation, flight efficiency and, ultimately, winter survival (Romero et al., 2005; Strochlic & Romero, 2008). Therefore, in theory, when the fitness costs of a low-quality moult outweigh those of not responding maximally to a stressor, the corticosterone stress response should be attenuated to ensure that the quality of moult is not compromised (Wingfield & Sapolsky, 2003). Therefore, these sex- and subspecies-differences in the corticosterone stress response may originate from the contrasted constraints that sexes and subspecies have to cope with during the moult period, such as habitat characteristics, predation risk or weather (Olsen et al., 2008, 2010).

Overall, these sex- and subspecies-differences in the stress response of swamp sparrows remain unclear because the ecology and the stress physiology of mouling swamp sparrows have been seldom studied in the wild (Mowbray, 1997; but see Greenberg et al., 2007). Specifically, the corticosterone stress response has never been measured and related to ecological or life-history variables in wild swamp sparrows. Therefore, further eco-physiological studies are needed to better understand the benefits and costs of mounting a strong stress response in mouling birds originating from different populations. This may help in understanding population-level constraints on stress response to global change.

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