

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

F. ANGELIER*[†]1, B. BALLENTINE*[‡], R. L. HOLBERTON§, P. P. MARRA* & R. GREENBERG*

*Smithsonian Migratory Bird Center, National Zoological Park, Washington, DC, USA

[†]Department of Neurobiology, Physiology and Behavior, University of California, Davis, CA, USA

[‡]Department of Biology, University of West Georgia, Carrollton, GA, USA

§Laboratory of Avian Biology, Department of Biological Science, University of Maine, Orono, ME, USA

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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.

Introduction

When unpredictable and life-threatening events occur, animals often mount a behavioural and physiological response to these stressors and this, in turn, activates an emergency life-history stage that redirects resources from activities nonessential to immediate survival towards processes that promote immediate survival (combating or escaping the stressor, Wingfield *et al.*, 1998). In vertebrates, endocrine processes are well known to be involved in the stress response and to activate this emergency life-history stage (Ricklefs & Wikelski, 2002; Wingfield *et al.*, 2008a; Angelier & Chastel, 2009). Thus, stressors induce a rapid elevation of circulating gluco-

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 moulting 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 (Holberton *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 & Holberton,

Correspondence: Frédéric Angelier, Smithsonian Migratory Bird Center, National Zoological Park, 3001 Connecticut Avenue NW, Washington, DC 20008, USA and Department of Neurobiology, Physiology and Behavior, University of California, Davis, CA 95616, USA.

Tel.: +33 5 49 09 96 19; fax: +33 5 49 09 65 26;

e-mail: frederic_angelier@yahoo.fr

¹Present address: Centre d'Etudes Biologiques de Chizé, CNRS, Villiers en Bois, F-79360, France.

2000; Wingfield & Sapolsky, 2003; Romero *et al.*, 2005; Lendvai & Chastel, 2008; Quillfeldt *et al.*, 2009).

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 & Droege, 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 all 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 × 9" D × 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 μ L) was collected from the alar vein with a 27-gauge needle. A second small sample (80 μ L) 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 (ANOVAS, $P > 0.500$ 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, moult 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* (ANOVAS, $P > 0.500$ 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 corticosterone levels measured during the moult. This project was approved by the Smithsonian Institution Animal Care and Use Committee (#2007-03).

Corticosterone assay

After collection, blood samples were immediately centrifuged 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 millilitres 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 models 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 indications that normality assumptions were violated. Therefore, 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 corticosterone 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:

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

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 of 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 ($\text{ng mL}^{-1} \text{min}^{-1}$). 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.

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).

Model	Biological assumption	Dependent variables	Independent variables
1	Effect of sex and subspecies on body condition	Body condition	Species, sex and interactions
2	Effect of sex, subspecies and condition on baseline corticosterone levels	Baseline corticosterone levels	Species, sex, body condition and interactions
3	Effect of sex, subspecies and condition on the corticosterone stress response	Stress-induced corticosterone levels	Species, sex, body condition and interactions

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 \pm SE: 1.92 ± 0.33 , Fig. 1).

Stress-induced corticosterone levels

In all individuals, 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

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.

Model	Dependent variables	Models	<i>K</i>	Log likelihood	AICc	Δ AICc
1	Body condition <i>n</i> = 32	Intercept	1	-44.91	91.95	0.00
		Sex	2	-44.49	93.40	1.45
		Subspecies	2	-44.83	94.07	2.12
		Sex, subspecies	3	-44.43	95.72	3.77
		Sex, subspecies, sex \times subspecies	4	-43.94	97.37	5.42
2	Baseline corticosterone level <i>n</i> = 32	Intercept	1	-64.54	131.21	0.00
		Condition	2	-63.64	131.69	0.48
		Subspecies	2	-64.48	133.37	2.16
		Sex	2	-64.53	133.48	2.27
		Condition, subspecies	3	-63.54	133.92	2.71
3	Stress-induced corticosterone level <i>n</i> = 32	Sex, subspecies, sex \times subspecies	4	-105.31	220.10	0.00
		Sex, subspecies, condition, sex \times subspecies	5	-105.24	222.80	2.70
		Subspecies	2	-110.02	224.46	4.36
		Sex, subspecies	3	-109.27	225.40	5.30
		Intercept	1	-115.70	233.54	13.44

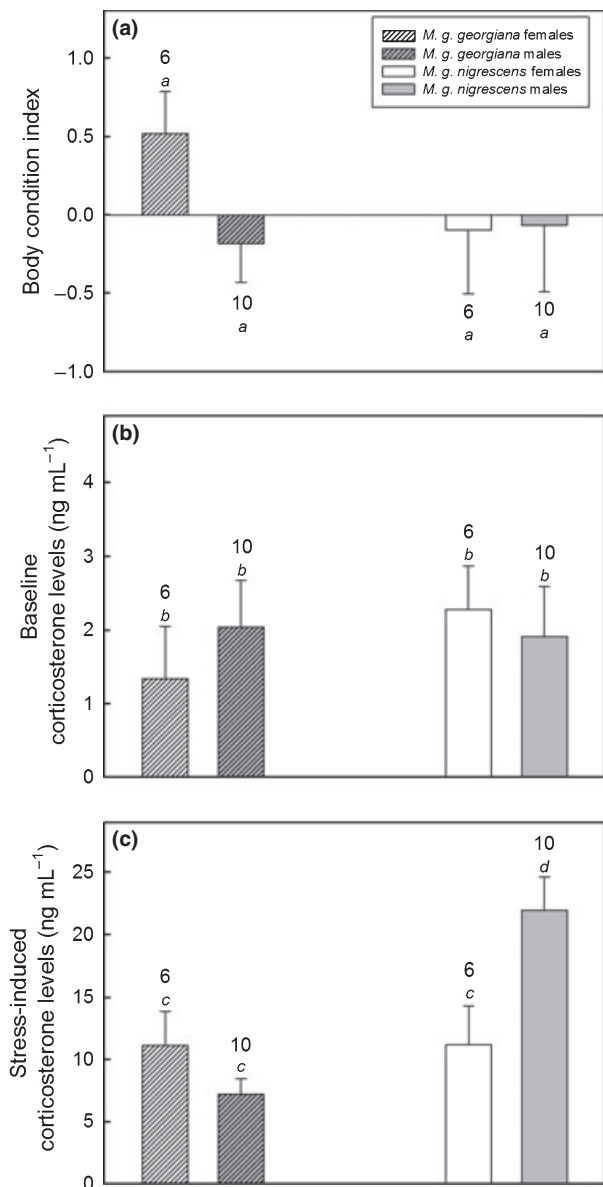


Fig. 1 Influence of subspecies (*Melospiza g. georgiana* vs. *M. g. nigrescens*) on (a) body condition, (b) baseline corticosterone levels and (c) stress-induced corticosterone levels in female and male swamp sparrows held in captivity for an extended period (common garden experiment). Error bars indicate SE and number above bars denote sample size. Letters above bars denote statistical results of comparisons between the different groups. The mean values of bars with the same letter do not significantly differ ($P > 0.05$) whereas the mean values of bars with different letters significantly differ ($P < 0.01$).

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).

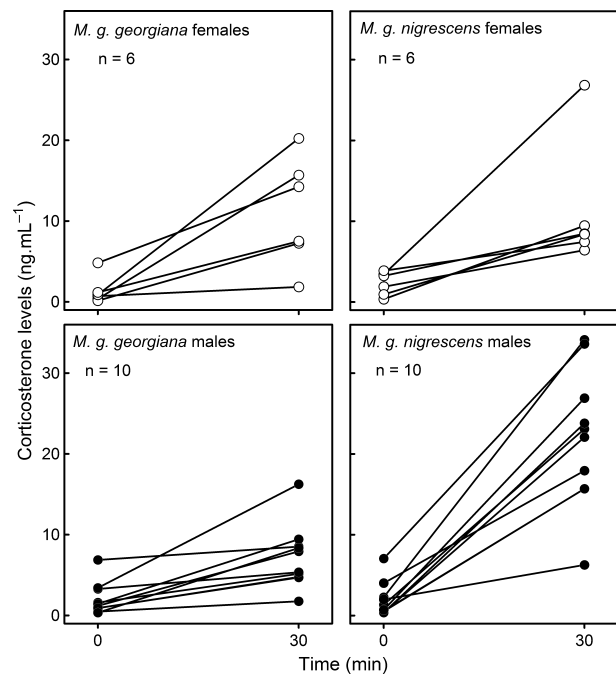


Fig. 2 Individual changes in corticosterone levels during a standardized handling stress protocol in two subspecies of swamp sparrows (*Melospiza g. georgiana*, *M. g. nigrescens*). Open symbols denote females and filled symbols denote males.

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 lose 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., 2005), 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 into 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; Strohlic & 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 moulting 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 moulting 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 moulting birds originating from different populations. This may help in understanding population-level constraints on response to global change.

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