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Stress and success: Individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk

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ABSTRACT

A fundamental element of how vertebrates respond to stressors is by rapidly elevating circulating glucocorticoid hormones. Individual variation in the magnitude of the glucocorticoid stress response has been linked with reproductive success and survival. But while the adaptive value of this response is believed to stem in part from changes in the expression of hormone-mediated behaviors, it is not clear how the behavior of stronger and weaker glucocorticoid responders differs during reproduction, or during exposure to ecologically relevant stressors. Here we report that in a population of barn swallows (Hirundo rustica erythrogaster) experiencing high rates of nest predation, circulating levels of corticosterone (the primary avian glucocorticoid) during exposure to a standardized stressor predict aspects of subsequent behavior and fitness. Individuals that mounted a stronger corticosterone stress response during the early reproductive period did not differ in clutch size, but fledged fewer offspring. Parents with higher stress-induced corticosterone during the early reproductive period later provisioned their nestlings at lower rates. Additionally, in the presence of a model predator stress-induced corticosterone was positively associated with the latency to return to the nest, but only among birds that were observed to return. Model comparisons revealed that stress-induced hormones were better predictors of the behavioral and fitness effects of exposure to transient, ecologically relevant stressors than baseline corticosterone. These findings are consistent with functional links between individual variation in the hormonal and behavioral response to stressors. If such links occur, then selection on the heritable components of the corticosterone stress response could promote adaptation to novel environments or predation regimes.

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Introduction

From taking shelter in extreme weather, to reducing parental behavior in the presence of predators, to coping with injury, the ways in which organisms respond to stressors can influence their likelihood of survival and reproduction (Wingfield and Kitaysky, 2002; Wingfield and Sapolsky, 2003). Glucocorticoid (GC) hormones (primarily corticosterone or cortisol) play a central role in orchestrating the response to stressful events in vertebrates (Sapolsky et al., 2000; Selye, 1946). When an organism is faced with an acute stressor GC secretion rises rapidly, triggering an increase in energy mobilization, combined with the suppression of behavioral and physiological processes that are not immediately essential to survival (Sapolsky et al., 2000; Wingfield and Romero, 2001). Individuals vary in circulating GC levels during exposure to an acute stressor (Cockrem, 2013a; Cockrem and Silverin, 2002), and this variation – which appears to have a heritable component (Evans et al., 2006; Pottinger and Carrick, 1999; Satterlee and Johnson, 1988; Touma et al., 2008) – has been linked with survival in several free-living populations. Stronger responders (with higher stress-induced GC levels) are more likely to survive in some environments (Angelier et al., 2009; Cabezas et al., 2007), but less likely to survive in others (Blas et al., 2007). The ability to rapidly terminate a stress response via negative feedback can also predict survival during selection events (Romero and Wikelski, 2010).

Stress responsiveness is believed to influence fitness in part through the differential performance of high- and low-responders during stressful events. Links between GC responsiveness and coping style have been suggested by research in captive populations (Baugh et al., 2013; Carere et al., 2010; Koolhaas et al., 1999, 2010), and studies in natural populations have found that the magnitude of the GC response can covary with several aspects of behavior including exploration and aggression (Kralj-Fišer et al., 2010; Lendvai et al., 2011; but see Garamszegi et al., 2012). Yet surprisingly little research has examined whether naturally strong GC responders also exhibit a stronger

behavioral response to acute stressors in the wild (Breuner et al., 2008; Cockrem, 2013b). Two recent studies provide some of the best evidence to date: young eiders (*Somateria mollissima*) that mount a stronger GC response also flee sooner from an approaching researcher (Seltmann et al., 2012), and great tits (*Parus major*) with higher stress-induced GCs during capture and restraint are more likely to abandon their first brood immediately following release (Ouyang et al., 2012). These findings support links between individual variation in the GC and behavioral response to human-induced threats; however, it is not clear whether individuals that mount a stronger GC response to a standardized stressor alter their behavior more when faced with ecologically relevant stressors that impact fitness.

Circulating GCs can influence reproductive success through several pathways. Chronically elevated 'baseline' GCs suppress many aspects of reproductive physiology and behavior, and extreme or prolonged elevations can induce complete reproductive failure (Sapolsky et al., 2000; Sheriff et al., 2009; Wingfield and Sapolsky, 2003). Even among individuals that successfully reproduce, those with higher baseline GCs, including during the early reproductive period, often have lower reproductive success (Angelier et al., 2010; Bonier et al., 2009a; Buck et al., 2007; Kitaysky et al., 2007; Love et al., 2004). However, moderate elevations in baseline GCs – which likely bind predominantly at mineralocorticoid receptors, rather than the glucocorticoid receptors that mediate the classic vertebrate stress response – can also stimulate parental behavior (Bonier et al., 2011; Crossin et al., 2012; Ouyang et al., 2013), and a positive association between baseline GCs and reproductive success has also been found in a number of populations (reviewed in Bonier et al., 2009a). The capacity or propensity to mount a GC response to acute stressors can also predict variation in reproductive success. Several studies have reported that individuals that mount a stronger GC response to standardized stressors have reduced reproductive performance and/or success compared with conspecifics that mount a more modest response (Husak and Moore, 2008; Lendvai and Chastel, 2010; Miller et al., 2009; Schmid et al., 2013; Vitousek et al., 2010a). These relationships often appear to be independent of variation in baseline GCs (Patterson et al., 2014), and, in some cases, stress-induced GCs are better predictors of reproductive behavior than baseline GCs, even though acute elevations in GCs are likely infrequent (Ouyang et al., 2012; Vitousek and Romero, 2013). Differences between stronger and weaker GC responders in behavior or fitness could result from a causal relationship between these factors, with higher hormone levels influencing reproductive phenotype either immediately or over more prolonged periods (e.g., carry-over effects) due to the cumulative effects of repeated or chronic GC elevation. Alternatively, these patterns could result from indirect links, with both GC levels and reproductive success being determined by other internal or external factors (e.g., body condition or environment). Regardless of the drivers of variation in stress responsiveness, differences in reproductive success between stronger and weaker responders could reflect variation in initial reproductive investment (e.g., the number of eggs or offspring produced), parental care (e.g., offspring provisioning rate), or correlated genetic differences in parents or offspring (e.g., susceptibility to disease, efficiency of energy conversion). Distinguishing among these possibilities will require detailed investigations of the links between stress responsiveness and reproductive performance across groups. Moreover, because the physiological state of stronger and weaker responders may differ only during or immediately following exposure to stressors, it is necessary to elucidate the context-dependency of links between stress responsiveness and reproductive performance.

Barn swallows (*Hirundo rustica erythrogaster*) breeding in Colorado, USA, periodically experience high rates of nest predation, with more than 50% of first attempts lost to predators in some years (Flynn, 2011). Nest predation therefore represents a strong selective force in this population. We tested whether natural variation in circulating corticosterone (the primary avian GC) was associated with reproductive

behavior and success. Specifically, we assessed whether the corticosterone response to a standardized capture-and-restraint stressor, or baseline circulating corticosterone levels during the early reproductive period, predicted: (1) parental behavior several weeks later (offspring provisioning rate and the behavioral response to a model predator at the nest), (2) initial reproductive investment (clutch size), and (3) variation in reproductive outcomes.

Because acute elevations in corticosterone may influence the expression of escape behavior (Thaker et al., 2010) and have been linked with nest abandonment (Schultner et al., 2013; Silverin, 1986), we predicted that individuals that mounted stronger GC responses (those with higher stress-induced corticosterone) would abandon nests at higher rates during laying and incubation, and would take longer to return to the nest in the presence of a predator. In contrast, we predicted that in the absence of a predator, provisioning rate would be unrelated to individual differences in stress-induced corticosterone. Although the relationship between baseline GCs and parental effort may be highly context-dependent (Bonier et al., 2009b; Jaatinen et al., 2013), under challenging conditions we would expect that individuals with higher baseline corticosterone during the nest construction and incubation phases would later provision nestlings at a lower rate.

Because nest predation is a major determinant of reproductive success in this population, we predicted that during years of high predation stress-induced corticosterone (which is individually repeatable in barn swallows: Vitousek et al., in preparation) would be more closely related to reproductive success than baseline corticosterone, which fluctuates according to energetic demand (Landys et al., 2006) and recent exposure to stressors (Malisch et al., 2010).

Methods

Capture and experimental methods

Barn swallows nesting at 17 sites in Boulder, Weld, and Jefferson Counties, Colorado (40°N, 105° W) were sampled during the nest building and incubation stages in May–July of 2010. Adults were captured on nests by hand, or by rapidly flushing them into mist nets; during this period both members of the pair typically roost on or near the nests. Blood samples (~30 μL each) were collected within 3 min of approach to assess baseline corticosterone (Romero and Reed, 2005) and again 15 min after initial disturbance and the onset of restraint, to measure stress-induced corticosterone (Breuner et al., 1999; Romero and Remage-Healey, 2000). All blood samples were collected from the brachial vein and placed on ice until they were returned to the laboratory. Plasma was separated from whole blood by centrifugation and stored at −70 °C until analysis. Body mass was measured with a Pesola spring balance, with birds placed in a cloth bag, and wing length measured to the nearest 0.25 mm. Birds of both sexes received color bands and USGS numbered bands. Following release, visual observations were used to identify banded adults as breeding at specific nests. Although barn swallows often raise multiple broods in a season, only the first brood of each pair was included in analyses. Clutch size was recorded as the maximum number of eggs observed in the nest at the same time; nests were checked every other day throughout the laying and incubation periods. Because of egg predation, which could influence individual eggs or the entire clutch, maximum clutch size was not always equivalent to the total number of eggs laid by the female.

Behavioral observations

Behavioral observations were conducted at the nest when nestlings were five days old (mean ± SE: 30.4 ± 1.9 days following capture and corticosterone measurements). The number of provisioning visits that each individual made to the nest was recorded for a period of 30 min by a single observer; observers were trained prior to data collection to

ensure consistency. A model predator – a house cat (Wildlife Treasures, Ely, MN), which induces high rates of predation in many songbirds (Loss et al., 2013) – was then introduced ~1.5 m (mean: 1.5 ± 0.03, range: 0.8–2.1 m) from the nest. Behavioral observations then resumed for an additional 30 min, during which time the visitation patterns of adults were again recorded. Because of structural variation among barns it was not always possible to place the model predator exactly the same distance from the nest. To account for this variation the distance from the model predator to each focal nest was included as a covariate in analyses (see below). Although more than one experimental nest was sometimes located within a single barn, typically only the target birds, whose nest was directly above the model predator, appeared to mount a behavioral response to the model predator. The behavioral response was limited to fleeing, circling, and alarm-calling; in no instances did the model predator elicit a mobbing or dive-bombing response from either the focal parents or other birds at the site.

Hormone assays

Corticosterone concentrations were estimated using enzyme immunoassay (EIA) kits (Enzo Life Sciences, Plymouth Meeting, PA, USA). Assay procedures were optimized for barn swallow plasma by stripping plasma samples with Norit-activated charcoal and then spiking these samples with a known amount of corticosterone (Wada et al., 2007, 2008). Tests were conducted across multiple dilution values (1:10, 1:20, 1:40, and 1:80) and concentrations of steroid displacement buffer (0%, 1%, and 2%), and revealed an optimal dilution factor of 1:40 with 2% steroid displacement buffer. All samples from each individual were assayed in duplicate on the same plate. Assay procedures were conducted according to the manufacturer's instructions, except that we included a six-standard curve on every plate. Inter- and intra-assay variation was 9.6% and 10.9%, respectively. All samples were above the detection threshold (0.30 ± 0.09 ng/mL).

Data analysis

Data analyses were conducted using SAS 9.2. Hormone measures were natural log-transformed to meet assumptions of normality and homogeneity. Baseline and stress-induced corticosterone were correlated, so the influence of these measures on behavior and fitness was modeled separately. Corticosterone levels during the early reproductive period did not differ between males (mean ± SE (ng/mL): baseline: 6.2 ± 0.7, $n = 38$; stress-induced: 37.6 ± 2.9, $n = 39$) and females (baseline: 6.3 ± 0.5, $n = 58$, $t = 0.15$, $P = 0.88$, Cohen's $d = 0.04$; stress-induced: 35.8 ± 2.4, $n = 58$, $t = 0.97$, $P = 0.33$, Cohen's $d = -0.21$). Among the birds that were subsequently identified as actively breeding and included in analyses of reproductive success, corticosterone levels did not differ between the nest building and laying/incubation periods in either males (baseline: build: 5.8 ± 1.2, $n = 13$, incubate: 6.0 ± 1.3, $n = 13$, $t = 0.66$, $P = 0.52$, Cohen's $d = -0.15$; stress-induced: build: 40.7 ± 19.2, $n = 13$, incubate: 32.4 ± 2.7, $n = 13$, $t = -1.33$, $P = 0.20$, Cohen's $d = 0.52$) or females (base-line: build: 6.4 ± 1.0, $n = 11$, incubate: 5.4 ± 0.8, $n = 16$, $t = -1.12$, $P = 0.27$, Cohen's $d = 0.43$; stress-induced: build: 40.5 ± 5.9, $n = 11$, incubate: 35.2 ± 4.0, $n = 16$, $t = -0.95$, $P = 0.35$, Cohen's $d = -0.37$). Additionally, analyses in this population indicate that circulating corticosterone levels are significantly individually repeatable across the nest building, incubation, and nestling provisioning phases (linear mixed model analyses using the restricted maximum-likelihood method (Lessells and Boag, 1987; Nakagawa and Schielzeth, 2010): baseline corticosterone: $R(SE) = 0.54(0.11)$, CI $= [0.29, 0.72]$, $P = 0.002$ stress-induced corticosterone: $R(SE) = 0.50(0.12)$, CI $= [0.25, 0.70]$, $P = 0.022$; Vitousek et al., in preparation).

Samples were collected within specified time windows (plasma: 21:00–01:00, behavior: 08:00-13:00) to minimize the effect of circadian rhythms on hormone and behavioral data. Baseline blood samples were collected within 3 min of disturbance; within this period corticosterone levels were unrelated to the latency between disturbance and blood sampling. Because Julian sampling date was significantly correlated with the timing of behavioral observations within the specified time window, we controlled for Julian date alone in analyses. If both parents at a nest had been sampled, one was randomly excluded from analyses of reproductive success to avoid pseudoreplication. Data on behavior and reproductive success were analyzed using generalized linear mixed models (GLMMs) with a Poisson distribution. Best-fit models of the predictors of reproductive success and parental behavior were identified using a corrected Akaike Information Criterion (AICc) for small sample sizes. The AICc weight, calculated for all models, provides an estimate of the relative probability of each model. All models were compared to a null model, and estimates and 95% confidence intervals are reported for all fixed effects.

GLMMs, used to examine the predictors of nestling provisioning behavior and the latency to return to the nest following the introduction of a model predator, included breeding site as a random effect. Because some nests used in behavioral trials were also part of a brood swap experiment in which brood size was increased or decreased by two chicks immediately after hatching (Jenkins et al., 2013), treatment group was included as a fixed effect in models of behavior. Clutch size data (the number of eggs) are included from all nests that proceeded to incubation, as the brood swap experiments did not begin until after eggs had hatched. Analyses of the number of nestlings fledged include only the nests in which the number of nestlings was not manipulated as a part of the brood-swap study. An information theoretic model comparison approach was used to evaluate models of behavior, which included corticosterone levels in combination with Julian date, sex, body condition (scaled body mass index: Peig and Green, 2009), and, in all models of behavior in the presence of a predator, the distance from the model predator to the nest. The relationships between circulating hormones and reproductive output were also modeled using GLMMs that included breeding site as a random effect and clutch initiation date, corticosterone, sex, scaled body mass index, and elapsed time (from blood sampling to clutch initiation), as fixed effects. There was no covariation among fixed effects.

Results

Corticosterone and parental behavior

The best-fit model of nestling provisioning rate prior to introducing the model predator included stress-induced corticosterone, scaled body mass index, and date (stressed cort: estimate [95% CI]: -0.48 [-0.83, -0.14], $F_{1,49} = 8.17$, $P = 0.006$, scaled body mass index: 0.08 [0.01, 0.15], $F_{1,49} = 5.05$, $P = 0.029$, date: 0.02 [0.00, 0.03], $F_{1,49} = 4.14$, $P = 0.047$, $n = 66$; Fig. 1). Parents with higher stress-induced corticosterone and lower body condition during the early reproductive period later provisioned their nestlings at lower rates.
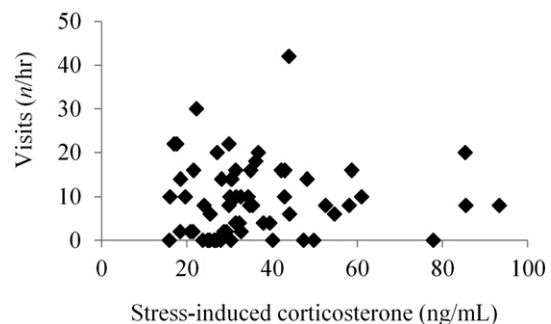

Fig. 1. Birds with higher stress-induced corticosterone during the early reproductive period later provisioned nestlings at lower rates.

After the model predator was introduced near the nest site, all adults ceased provisioning their nestlings for a period of time. Females were more likely than males to return to the nest before the predator trial concluded, but many individuals did not return until the model was removed (females: $n = 20$ returned, $n = 19$ not returned; males: $n = 6$ returned, $n = 21$ not returned). When all individuals were included in the model, with a minimum latency time of 30 min substituted for all non-returners, sex was the only significant factor in the best fit model of return latency (Table 1; stressed cort: -0.15 [$-0.31, 0.02$], $F_{1,49} = 3.11$, $P = 0.08$, predator distance: -0.07 [-0.14, 0.00], $F_{1,49} = 3.62$, $P = 0.06$, sex: 0.22 [0.11, 0.33], $F_{1,49} = 15.90$, $P < 0.001$, date: 0.003 [$-0.001, 0.011$], $F_{1,49} = 2.62$, $P = 0.11$; $n = 66$). When analyses were conducted using only the individuals that did return to the nest during the period of observation stress-induced corticosterone, along with sex, treatment, and the distance from the model predator to the nest were significant predictors of the latency to return in the best-fit model (Table 1; stressed cort estimate: 0.56 [0.12, 0.99], $F_{1,11} = 7.99$, $P = 0.017$, predator distance: -0.47 [$-0.67, -0.28$], $F_{1,11} = 27.54$, $P < 0.001$; sex: 0.51 [0.09, 0.92], $F_{1,11} = 7.50$, $P = 0.019$, treatment: control: 0.40 [0.02, 0.78], decrease: -0.20 [$-0.57, 0.18$], $F_{2,11} = 4.76$, $P = 0.032$; $n = 26$). Of the birds that returned to the nest during the trial, those that produced more corticosterone in response to the standardized stressor during the early reproductive period exhibited a stronger behavioral response to a model predator during the nestling provisioning phase, taking longer to return to the nest to resume provisioning. Models that included baseline corticosterone were a poorer fit to the behavioral data than those that included stress-induced corticosterone.

Corticosterone and reproductive success

Clutch size was not predicted by any of the variables we measured; the null model was a better fit to the data than any other model we assessed (Table 1). The second-ranked model of clutch size, which included stress-induced corticosterone alone, had a substantially lower weight (0.11 vs. 0.31 for the null model; Fig. 2a; $n = 50$). Nest abandonment was rare, but the few females that did abandon clutches prior to the onset of incubation had significantly higher baseline and stress-induced corticosterone prior to abandonment than those that did not (baseline: $t = -3.46$, $P = 0.009$, Cohen's $d = 1.08$, stress-induced: $t = -7.8$, $p < 0.0001$, Cohen's $d = 1.99$; $n = 50$ non-abandoning females, $n = 3$ abandoning females).

The best-fit model of the number of nestlings that fledged from a given nest included stress-induced corticosterone and clutch initiation date (stressed cort: -3.25 [$-5.72, -0.78$], $F_{1,5} = 11.42$, $P = 0.020$; date: -0.05 [$-0.12, 0.01$], $F_{1,5} = 4.21$, $P = 0.10$; $n = 16$, Fig. 2b).

Table 1
Models of the predictors of reproductive success and behavior in breeding barn swallows. The best-fit model and models <2 ΔAICc are shown, as are all null models.

| Model	AICc	ΔAICc	K	Weight
Provisioning rate				
Stressed cort + condition + date	376.08		4	0.19
Stressed cort + condition	377.88	1.8	3	0.08
Null model	385.52	9.44	1	0.00
Latency to return – predator – all individuals				
Stressed cort + model dist + sex + date	728.27		5	0.28
Stressed cort + model dist + sex	728.47	0.2	4	0.25
Stressed cort + model dist + sex + treat	729.17	0.9	5	0.18
Null model	740.96	12.69	1	0.00
Latency to return – predator – returners only				
Stressed cort + model dist + sex + treat	210.45		5	0.29
Stressed cort + model dist + date	212.25	1.8	4	0.12
Null model	242.33	31.88	1	0.00
Clutch size				
Null model	176.6		1	0.31
Fledging success				
Stressed cort + date	57.47		3	0.55
Null model	71.12	13.65	1	0.00
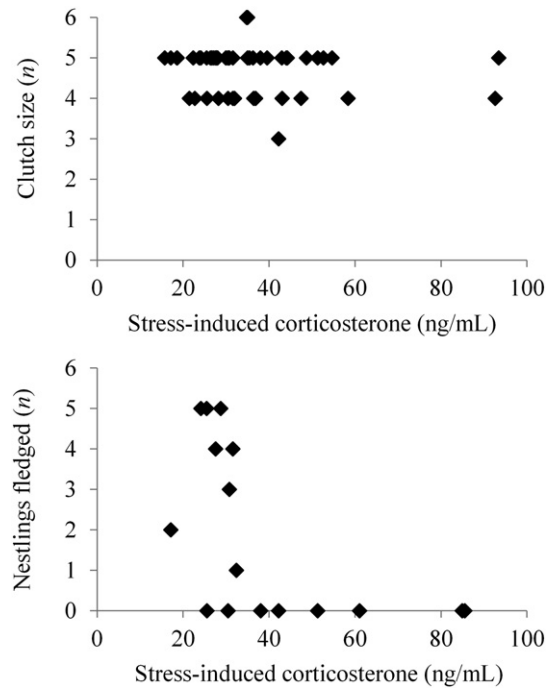
Fig. 2. Corticosterone and measures of reproductive success. (a) Stress-induced corticosterone was unrelated to completed clutch size. (b) Adults with lower stress-induced corticosterone subsequently fledged more offspring.

Birds with higher stress-induced corticosterone during the early reproductive period therefore fledged fewer young. Models that included baseline corticosterone were a substantially poorer fit to the data on both measures of reproductive success.

Discussion

Stress-induced corticosterone, behavior, and reproductive success

Individual variation in circulating corticosterone during exposure to a standardized acute stressor predicted reproductive behavior, elements of the behavioral response to a predator during the nestling period, and the ultimate fledging success of free-living barn swallows. In contrast to our prediction that baseline corticosterone would be a better predictor of provisioning rate than stress-induced corticosterone, the best-fit model of parental provisioning behavior revealed a negative association between stress-induced corticosterone during the early reproductive period and subsequent nestling provisioning rate. In this study corticosterone levels were measured several weeks prior to the nestling period, and thus may not have accurately reflected circulating hormone levels during later stages of reproduction; however, other data from this population suggest that variation in corticosterone levels is heritable (Jenkins et al., 2014) and that circulating corticosterone is individually repeatable during the reproductive period (Vitousek et al., in preparation; see above). Lower stress-induced corticosterone levels are often seen in individuals and groups investing more in reproduction (Holberton and Wingfield, 2003; Lendvai et al., 2007; Schmid et al., 2013; Vitousek et al., 2010a; Vitousek and Romero, 2013), and could result from increases in corticosterone directly mediating reproductive behavior, or from non-causal associations between physiological and behavioral phenotype. Because mounting an acute stress response is relatively uncommon in many systems it is not always clear how variation in stress-induced hormone levels could mediate the expression of reproductive behavior under normal conditions. However, during the year of study conditions were particularly poor due to both high rates of nest predation and periods of extreme temperatures (low in the

early season and high in the mid-late season). Brief stress-induced increases in circulating corticosterone may therefore have been relatively frequent during the study period, and thus could potentially have directly influenced the expression of reproductive behavior.

Maintaining a robust acute stress response is believed to enable an organism to respond more effectively to current and future stressors, including the threat of predation (Sapolsky et al., 2000; Selye, 1937; Thaker et al., 2010; Wingfield et al., 1998). However, while GC responsiveness has been linked with the response to novel objects and human activity (Baugh et al., 2013; Carere et al., 2010; Ouyang et al., 2012; Seltmann et al., 2012), it is not clear whether stronger GC responders show a greater behavioral response to ecologically relevant acute stressors under natural conditions (reviewed in Breuner et al., 2008; Cockrem, 2013b). In this study, stress-induced corticosterone levels were predictive of some aspects of behavior during exposure to stressors. When presented with a model predator near the nest, barn swallow parents ceased provisioning five-day-old nestlings for a period of time, and many individuals remained away from the nest for the duration of the thirty-minute experimental predator presentation. When non-returners were included in the analysis (using an artificial minimum latency time of 30 min for the non-returners), sex was the only significant predictor of the latency to return: females resumed provisioning more quickly than males. The distance from the model predator to the nest and stress-induced corticosterone during the incubation period were not significant predictors of return latency, but both showed a negative trend. However, the ability to draw conclusions from a mixed model analysis of this data is likely quite limited due to the high proportion of non-returners (~60%). Analyses that were conducted using only birds for which we had measured return latencies (those that returned in less than 30 min) indicated that the latency to return to the nest was positively predicted by stress-induced corticosterone during the early reproductive period, and negatively associated with the distance from the model predator to the nest. In other words, among individuals that returned to the nest in the presence of the predator, those that exhibited a stronger corticosterone response also exhibited a stronger behavioral response to the presence of a predator, remaining away from the nest for longer prior to returning.

As all birds returned to the nest after the predator trials were complete, a longer trial would likely have enabled us to observe a higher proportion of returners, and in turn improved our ability to determine the physiological predictors of the latency to return among all birds and to assess the potential for interactions among the variables of interest. Additionally, while corticosterone levels are individually repeatable during the reproductive period in barn swallows, measurements of the stress response taken closer to the period of behavioral measurement, or in response to a similar type of stressor (e.g., model predator vs. restraint stress) might be a more precise reflection of the physiological state of individuals undergoing a predator challenge. Our results do, however, suggest that stress-induced corticosterone may be differentially related to return latency among birds that did and did not return to the nest during the period of observation. We did see some indications that the behavior of returners and non-returners may have differed throughout the trial. Birds that returned to the nest following the predator presentation often remained nearby, circling and alarm-calling for a period of time before returning to the nest itself. In contrast, adults that did not return were typically not observed in the vicinity of their nesting site for the duration of the trial. Thus, in some individuals, the initial exposure to a predator appears to have resulted in the decision to redirect behavior away from the nest for an extended period. The behavioral response to predators may therefore proceed as a two-step process in barn swallows, with the first step – leave for an extended period or remain nearby – mediated by other physiological (e.g., catecholamines) or life history (e.g., age, breeding history) factors.

In vertebrates, the behavioral stress response is influenced by both GC and catecholamine hormones, but the relative roles of these hormones in mediating specific aspects of behavior under different contexts are poorly understood. Catecholamines, which rise rapidly following exposure to stressors, mediate the activation of the sympathetic nervous system, and influence some aspects of the immediate behavioral response to stress. Free-living Galápagos marine iguanas injected with the catecholamine epinephrine – but not corticosterone – flee sooner from a novel predator than their control-injected counterparts (Vitousek et al., 2010b). But while GCs do not begin to rise for several minutes after the onset of a stressor, the activation of the hypothalamic–pituitary–adrenal axis can rapidly influence behavior through several pathways, including the direct behavioral effects of upstream components of this response (e.g., corticotropin-releasing hormone: Smagin et al., 2001; Sutton et al., 1982), and through GCs altering the memory of and subsequent behavioral response to stressors (Roozendaal et al., 2006). Individuals that are prevented from mounting a GC response via pharmacological blockade mount a weaker behavioral response to stressors (reduce flight initiation distance: Thaker et al., 2010) and are less able to retain information about threats and modulate the response to future stressors accordingly (Akirav et al., 2004; Thaker et al., 2010). Elucidating the relative roles of these hormones in mediating different aspects and stages of the behavioral stress response could help to reveal both the constraints on the behavioral response to stressors, and the potential for selection to shape the heritable components of these traits.

Clutch size was unrelated to circulating hormones, or to any other variables we measured, indicating that birds that mounted a stronger corticosterone stress response did not differ in fledging success as a result of differences in the number of eggs laid. However, although sample sizes were low, we saw some evidence that birds that mounted a stronger stress response were more likely to abandon nesting attempts prior to the onset of incubation than those that had previously mounted a weaker hormonal stress response. These results are similar to findings in great tits (*P. major*), where males with higher stress-induced corticosterone levels are more likely to abandon nests during poor conditions (Ouyang et al., 2012).

Our analyses of fledging success are based on a lower sample size than those of clutch size, but the results are striking: not a single individual that had stress-induced corticosterone levels above 35 ng/mL during the early reproductive period subsequently fledged offspring in the first clutch (Fig. 2b), despite the fact that this level was equivalent to the population mean (36.5 ± 1.8 ng/mL; Fig. 2a). Clutch initiation date, which is commonly associated with reproductive success among passerines (Verhulst and Tinbergen, 1991; Winkler and Allen, 1996) including some populations of barn swallows (Møller, 1992; Safran, 2004), was also included in the best-fit model, although this factor did not reach significance. Nest predation that occurs randomly or sporadically could alter the common pattern whereby more experienced breeders and/or individuals of higher phenotypic quality initiate clutches earlier in the season and achieve higher reproductive success. Yet despite the possibility of random or sporadic nest predation obfuscating the relationship between clutch initiation date or phenotypic traits and reproductive success, we saw consistent and strong relationships between corticosterone levels, nest abandonment, and reproductive success. These findings support the possibility that nest failure and offspring mortality were biased toward the nests of parents that secreted more corticosterone in response to stress. If the nestlings of high corticosterone responders were unattended for a longer period of time in the presence of predators or other naturally occurring stressors, they may have been more susceptible to predation, or received less food overall as a result of repeated parental absences. Alternatively, the lower survival rates at the nests of parents with higher stress-induced corticosterone could result from the reduced overall provisioning rate at these nests.

Baseline corticosterone

Baseline corticosterone levels during the incubation period were not significant predictors of subsequent parental behavior or reproductive

success in barn swallows. Because baseline GCs can fluctuate based on both energetic demand and exposure to stressors, the relationship between baseline GCs, behavior, and fitness is likely highly context-dependent (Bonier et al., 2009a; Jaatinen et al., 2013). When resources are plentiful, moderate elevations in circulating GCs may facilitate nestling provisioning by promoting energy mobilization (Bonier et al., 2009b, 2011; Crossin et al., 2012; Ouyang et al., 2013). But because GCs are also elevated in individuals responding to events that they perceive as stressful, higher baseline levels may also be seen in parents struggling to cope with the demands of surviving and reproducing under poor conditions (Jaatinen et al., 2013; Sheriff et al., 2009). The lack of an association between baseline corticosterone and behavior in models of reproductive success may result from shifting patterns between the incubation and nestling periods, or from non-linear relationships between corticosterone and fitness. It is intriguing, however, that in this study the capacity to respond to stressors (as measured by corticosterone levels during exposure to a standardized stressor) was a substantially better predictor of subsequent behavior and reproductive success than baseline hormone levels.

Conclusions

The observed links between corticosterone responsiveness, behavior, and reproductive success indicate that the hormonal response to restraint stress can, at least under some conditions, be a reliable predictor of both performance and components of fitness in free-living populations. Intriguingly, the predictive power of circulating hormones persisted over time and across life history sub-stages. Individual variation in the regulation of GC levels is likely influenced by a combination of environmental and genetic factors. Stress-induced corticosterone levels are moderately to highly heritable in this population of barn swallows (Jenkins et al., 2014) which is in accordance with findings of heritability in stress-induced GCs from selection studies in other species (Evans et al., 2006; Pottinger and Carrick, 1999; Satterlee and Johnson, 1988; Touma et al., 2008). Moreover, artificial selection studies have found that selection acting on either stress-induced GC levels or on the behavioral response to challenges can cause correlated changes in both traits (Baugh et al., 2012; Pottinger and Carrick, 2001; Stöwe et al., 2010). Taken together, these findings suggest the potential for selection to act directly or indirectly on heritable variation in stress-induced GC levels to shape the integrated phenotypic response to challenges.

A key priority for future research is establishing whether and under what conditions individual variation in GC levels during acute stress directly mediates the behavioral response to challenges and reproductive investment (Crespi et al., 2013). Experimental tests in which hormone levels are manipulated in free-living populations to accurately mimic acute, transient increases in GCs – rather than the chronic elevations typically produced – are needed to assess the potential mechanisms linking the response to stress with behavior and fitness. Acute, transient increases in GCs could alter the likelihood of engaging in specific adaptive behaviors that influence reproductive success. For example, as tested here, individuals that are more responsive to stressors could refrain from reproductive activities for a longer period when faced with a stressor, which might result in a greater likelihood of reproductive failure. Greater elevations in GCs during the course of an acute stress response, particularly if multiple stress responses are induced over a short time period, could also alter the 'reactive scope' (the normal physiological range of an individual: Romero et al., 2009) by inducing 'wear and tear.' The resulting decrease in the threshold between reactive homeostasis and homeostatic overload would increase the likelihood that an individual enters a state of homeostatic overload. In this way, consistent differences in hormone responses during one life history stage could lead to carry-over effects on behavior or phenotype in subsequent life history stages. Alternatively, individuals planning to invest heavily in reproduction might down-regulate the GC response prior to reproduction to minimize the potential impact of exposure to acute stressors (Wingfield and Sapolsky, 2003). In this case, the 'pre-emptive' down-regulation of the GC response could result in correlations between hormones, behavior, and fitness without circulating GCs directly mediating reproductive effort. Experimental manipulations that test whether acute and transient increases in GCs – similar to those experienced by organisms in the presence of short-duration stressors — decrease immediate or future reproductive investment (Busch et al., 2008; Ouyang et al., 2012), and whether these relationships scale with natural variation in the magnitude of the GC response (Lendvai and Chastel, 2010), will help to elucidate these mechanisms.

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