



## Stress responsiveness predicts individual variation in mate selectivity

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### ABSTRACT

Steroid hormones, including glucocorticoids, mediate a variety of behavioral and physiological processes. Circulating hormone concentrations vary substantially within populations, and although hormone titers predict reproductive success in several species, little is known about how individual variation in circulating hormone concentrations is linked with most reproductive behaviors in free-living organisms. Mate choice is an important and often costly component of reproduction that also varies substantially within populations. We examined whether energetically costly mate selection behavior in female Galápagos marine iguanas (*Amblyrhynchus cristatus*) was associated with individual variation in the concentrations of hormones previously shown to differ between reproductive and non-reproductive females during the breeding season (corticosterone and testosterone). Stress-induced corticosterone levels – which are suppressed in female marine iguanas during reproduction – were individually repeatable throughout the seven-week breeding period. Mate selectivity was strongly predicted by individual variation in stress-induced corticosterone: reproductive females that secreted less corticosterone in response to a standardized stressor assessed more displaying males. Neither baseline corticosterone nor testosterone predicted variation in mate selectivity. Scaled body mass was not significantly associated with mate selectivity, but females that began the breeding period in lower body condition showed a trend towards being less selective about potential mates. These results provide the first evidence that individual variation in the corticosterone stress response is associated with how selective females are in their choice of a mate, an important contributor to fitness in many species. Future research is needed to determine the functional basis of this association, and whether transient acute increases in circulating corticosterone directly mediate mate choice behaviors.

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### 1. Introduction

Steroid hormones are often implicated in cueing the rapid behavioral and physiological shifts associated with transitions among life history states (Adkins-Regan, 2005; Ketterson and Nolan, 1992; Wingfield et al., 1998). Changes in circulating hormone concentrations may also play a role in mediating trade-offs within life history stages, and in regulating components of reproductive investment (Wingfield et al., 1990, 1998). For example, in males of many avian species, an increase in testosterone promotes behaviors associated with mate and territory acquisition, while down-regulating parental care (Hau, 2007; Ketterson and Nolan, 1999).

Glucocorticoid hormones, which have widespread pleiotropic effects, may also influence investment in life history stages. Sub-

stantially elevated circulating glucocorticoids are hypothesized to promote survival during an acute stress response by up-regulating energy mobilization while simultaneously suppressing processes that are not necessary for immediate survival, including reproduction (Sapolsky et al., 2000; Wingfield and Sapolsky, 2003). Circulating corticosterone (the primary glucocorticoid in birds, reptiles, amphibians, and rodents) rises sharply prior to nest abandonment in breeding birds faced with challenging environmental conditions (Love et al., 2004; Wingfield and Kitaysky, 2002), and the experimental administration of exogenous glucocorticoids reduces parental behavior in many species (Angelier et al., 2009a; Horton and Holberton, 2009; Kitaysky et al., 2001), with supraphysiological levels inducing reproductive failure (O'Connor et al., 2009; Silverin, 1986). While seasonal and geographical patterns in corticosterone secretion are not well understood (Hau et al., 2010; Romero, 2002), comparative work indicates that the corticosterone stress response (the increase in corticosterone secreted in response to a stressor) may be relatively weaker during reproduction in populations with temporally constrained reproductive seasons, and in those breeding at high latitudes, to avoid the deleterious effects of

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high corticosterone on reproduction (Eikenaar et al., 2012; Holberton and Wingfield, 2003; Wingfield and Sapolsky, 2003; but see, Breuner et al., 2003). A phylogenetic comparative analysis found that the corticosterone stress response is weaker during breeding in avian species where a single clutch represents a higher proportion of lifetime reproductive effort (Bókony et al., 2009; but see, Breuner, 2010).

While the available evidence suggests that substantially elevated glucocorticoid levels – which bind predominantly to glucocorticoid receptors – negatively impact reproduction, the relationship between baseline glucocorticoids and reproductive investment is less clear. In non-chronically or acutely-stressed individuals, baseline corticosterone binds predominantly to mineralocorticoid receptors that play a major role in metabolic regulation (Landys et al., 2006). Moderately elevated baseline glucocorticoid levels might therefore promote energetically costly reproductive processes up to a point; however, if baseline corticosterone is elevated to a degree that substantially increases glucocorticoid-receptor binding, then a negative relationship between circulating corticosterone and reproductive behaviors or success may result (Bonier et al., 2009; Romero et al., 2009).

Within-populations, individuals vary dramatically in circulating hormone levels (Williams, 2008), and this variation can predict subsequent reproductive success and survival (Angelier et al., 2009b; Blas et al., 2007; Cabezas et al., 2007; Husak and Moore, 2008; Romero et al., 2009; Romero and Wikelski, 2010). However, the relationship between individual variation in glucocorticoid levels and most reproductive behaviors in free-living individuals is poorly understood. Some of the strongest evidence to date is of a link between parental behavior and stress-induced corticosterone. In several species of birds, parents that mount a stronger corticosterone stress response during the early breeding season subsequently provision their offspring at lower rates (Lendvai and Chastel, 2010; Miller et al., 2009; Ouyang et al., 2011b). Similarly, in at least some species, the corticosterone stress response may itself be influenced by potential reproductive gain: breeding house sparrows (*Passer domesticus*) with experimentally increased brood sizes secrete less corticosterone in response to a stressor (Lendvai et al., 2007). The presence or direction of a relationship between natural variation in baseline corticosterone and parental behavior is less clear (Ouyang et al., 2011b; Wilcoxon et al., 2010; Bonier et al., 2011; Lendvai et al., 2007), and little is known about whether other aspects of reproductive behavior are associated with individual variation in circulating corticosterone.

Mate choice is an important component of reproduction that can influence fitness (Andersson, 1992). The process of mate selection may incur substantial costs (Byers et al., 2005; Daly, 1978; Vitousek et al., 2007) that fluctuate based on intrinsic and extrinsic factors, including population structure, environmental conditions, health, and life history stage. Choosers alter both mate preferences and mate selectivity (the strength of preferences) in response to changes in the cost of selection (Byers et al., 2006; Jennions and Petrie, 1997; Milinski and Bakker, 1992). Despite the plasticity and fitness consequences of mate choice, little is known about the physiological mediators of mate selectivity. In what is to our knowledge the only study to examine the effect of an acute glucocorticoid increase on mate choice behavior, female mice administered exogenous corticosterone showed a marked dose-related decrease in their preference for male odors (Kavaliers and Osseknopp, 2001). Experimental treatment with exogenous testosterone (McGlothlin et al., 2004), estrogen (Vyas et al., 2009) and human chorionic gonadotropin (Lynch et al., 2006) can also influence aspects of female mate choice behavior, but as far as we are aware, the relationship between individual variation in hormone secretion and mate selectivity has not been examined in any species.

Here we use a two-year dataset of physiological and behavioral measures [described in Vitousek et al., 2010] to investigate the hormonal predictors of mate selectivity – defined as the total number of unique males assessed by each female – in female Galápagos marine iguanas (*Amblyrhynchus cristatus*). During the breeding season, the largest male iguanas hold resource-independent display territories, and court visiting females with a testosterone-mediated head-bob display (Wikelski et al., 2005). Females visit the territories of many males over an approximately 45-day period (Vitousek et al., 2007), and ultimately select a mate based on his rate of head-bob display (Vitousek et al., 2007, 2008; Wikelski et al., 2001). There is no evidence of mate-choice copying in this system: females make independent mating decisions based on the information that they gather about the head-bob rate of prospective mates during the same time period that we examine here (Wikelski et al., 2001). Reproductive female marine iguanas incur significant energetic costs during reproduction that result from both traveling among territorial males and responding to male displays (Vitousek et al., 2007). Males approach females closely while conducting side-walking head-bob courtship displays; females that are not interested in copulating move away from the displaying male, incurring the resulting energetic cost. Reproductive skew is high: females most often mate with only one male per season, but successful males can copulate with more than 20 females (Wada et al., 2008; Wikelski et al., 1996, 2001).

Female marine iguanas reproduce, on average, biennially (Laurie, 1990), and the decision whether to reproduce in a given year is strongly predicted by individual variation in the corticosterone stress response (Vitousek et al., 2010). Reproductive females maintain much lower levels of both baseline and stress-induced corticosterone for the duration of the breeding season (Vitousek et al., 2010). Among reproductive individuals, substantial inter-individual variability exists in both mate selection behaviors (Vitousek, 2009; Vitousek et al., 2007) and circulating hormone concentrations (Vitousek et al., 2010). Female marine iguanas assess between 1 and 25 males per season, and those that assess more territorial males ultimately mate with a more successful male (defined as a male receiving a greater proportion of the total annual copulations (Vitousek, 2009). Particularly selective females face increased energetic expenditure on mate choice behaviors, lose more mass during mate selection, and develop smaller eggs (Vitousek et al., 2007). Hormone concentrations are also highly variable among individuals, with circulating corticosterone and testosterone ranging over 20-fold among reproductive females alone (Vitousek unpublished data). Here we examine whether, among reproductive females, individual variation in the titers of corticosterone and testosterone is associated with individual variation in costly mate selection behavior.

Because of the deleterious effects of high corticosterone levels on reproduction, we predicted a negative relationship between stress-induced corticosterone during the early breeding season and subsequent mate selectivity. We also predicted that females with greater scaled mass indices (Peig and Green, 2009) will be more selective about potential mates because of the high energetic cost of mate selection behaviors (Vitousek et al., 2007). The presence and nature of a relationship between mate selectivity and baseline corticosterone or testosterone is more difficult to predict because of a comparative lack of relevant conceptual and empirical studies in other taxa. However, because resource availability was fairly typical during the years of study, and most individuals are not likely to have been experiencing chronic stress, we tentatively predicted that baseline corticosterone was either unrelated or positively related to mate selectivity. We also predicted a negative association between testosterone and mate selectivity, as has been revealed by experimental work in a songbird (McGlothlin et al., 2004).

Because older females have fewer opportunities for reproduction, we predicted that older reproductive females would maintain lower stress-induced corticosterone (and potentially lower circulating testosterone) than younger individuals both during the breeding period and immediately post-breeding/pre-laying. Finally, if circulating hormone concentrations play a role in mediating specific components of reproductive investment, we predicted that hormone concentrations would be individually consistent within the breeding season (prior to and during mate selection), but not necessarily between reproductive stages (breeding vs. post-breeding).

## 2. Material and methods

### 2.1. Sampling and measurements

Reproductively active adult-sized females were captured (by hand or with the aid of a loop attached to a bamboo pole) at Bahia Paraiso on Isla Santa Fe, Galápagos, Ecuador (90 2'W, 0 50'S) from November–January in 2003–04 and 2004–05. Baseline blood samples were collected from the caudal or jugular vein within 3 min of approach and capture to determine circulating corticosterone and testosterone levels. In marine iguanas, glucocorticoid levels do not begin to rise for at least 3 min following the onset of a stressful stimulus (Romero and Reed, 2005), and peak within 30 min (Romero and Wikelski, 2001). Females were placed in a cloth bag for 30 min, at which point a second blood sample was taken to determine stress-induced corticosterone levels. This standardized restraint stress protocol measures the ability of the hypothalamic–pituitary–adrenal axis to respond to a stressor (Romero, 2004), and has been used extensively in marine iguanas (Romero and Wikelski, 2001, 2002; Vitousek et al., 2010). Due to logistical constraints captures could not always be conducted at the same time of day; however, during the breeding season baseline corticosterone is only significantly elevated in females around mid-day (Woodley et al., 2003). Thus, we avoided sampling at mid-day (between 10:00 and 14:00 h). All protocols were approved by Princeton University's Animal Care and Use Committee (IACUC #1439).

At each capture, snout–vent length was recorded to the nearest 1 mm and body mass measured to the nearest 10 g using a 1000 g Pesola spring balance. Females were marked with a number painted on their flank using nontoxic paint, a method that does not affect social interactions (M. Wikelski and D. Audet, unpublished data), and released at the capture site. All males holding territories in the area were also captured and marked to enable individual identification.

The complete sampling schedule for the individuals included in the study has been published previously (Vitousek et al., 2010). Briefly, we maintained two study sites at adjacent areas along the coastline. Females in both groups were captured, sampled, and measured during the period when males were beginning to initiate territories ('early breeding season'), and prior to the start of any copulations at the study sites (Nov 10–29). Females in both groups were recaptured and resampled immediately after the mate selection period ('post-breeding'), when all copulations on the territory had ceased, and females were preparing to leave the coastline for the nesting grounds (Dec 30–Jan 8). During the intervening period (Nov 30–Dec 29; 'mate selection'), the sampling protocol at the two sites differed. At one study site ( $n = 21$  reproductive females), extensive behavioral observations (see below) were conducted on all study females, but recaptures were not conducted to avoid disturbing behavior. Females at the other site ( $n = 16$  reproductive females) were targeted for recapture and resampling every ten days throughout the mate selection period. Detailed behavioral observations were not conducted at this site.

Reproductive state was assigned by observing copulations and palpating the abdominal cavity of females during the final capture to check for the presence of developing eggs. In the second year of study (2004–05) the presence of vitellogenic eggs in reproductive females was confirmed with a PharVision Palm Scan Ultrasound (Classic Digital Imaging, Tequesta, FL, USA). Non-reproductive females showed a clear degeneration of follicles marked by a reduction in echogenicity (appearing as a blurring and lightening) of follicular structure and contents. Only data from reproductive females are used in this study.

### 2.2. Behavioral observations

Marine iguanas are extremely site-faithful (Romero and Wikelski, 2010; Wikelski and Trillmich, 1994) and their breeding behavior occurs in the open, which enables the observation of territory visitations, copulations, foraging, and basking behavior. Focal behavioral samples were conducted on reproductive females at one of our two study sites ( $n = 21$  reproductive females; 9 in 2003–04 and 12 in 2004–05). All individuals were studied for a single season. Observations were conducted continuously during all daylight hours throughout the mate selection period (06:00–18:00 h, which represents the total active period (Wikelski and Hau, 1995) by two concurrent observers who noted female location (on territory, off territory, in intertidal foraging zone), and the identity of the territorial males visited. Simultaneous focal observation of multiple individuals is possible due to the low activity level of female marine iguanas.

### 2.3. Hormone analyses

Blood samples were spun in the field with a homemade battery powered centrifuge (courtesy of Art deVries, University of Illinois). Plasma was extracted and stored in 750  $\mu$ L of 99.5% ethanol until it could be transported to Princeton University and stored at  $-20^{\circ}\text{C}$ . Storage in ethanol has been shown to be a valid means of preserving plasma samples in the field (Goymann et al., 2007; Tarlow et al., 2003). Plasma was extracted from ethanol using dichloromethane and reconstituted in 200  $\mu$ L of phosphate buffered saline with gelatin (PBSG buffer). Radioimmunoassays were conducted according to previously described protocols (Wingfield and Farner, 1975), using corticosterone antibody B3-163 (Esoterix Endocrinology, Calabasas, CA), and testosterone antibody T-3003 (Wiens Laboratory, Succasunna, NJ). All samples were assayed in duplicate using 50  $\mu$ L of plasma. All samples from each individual were run in the same assay, with individuals randomly distributed across assays. Each assay included three replicate standards. Intra-assay variation was 8% for corticosterone (over four assays) and 9% for testosterone (five assays); inter-assay variation was 12% for corticosterone and 25% for testosterone. Detection limits were 0.35 ng/mL for corticosterone and 0.19 ng/mL for testosterone; undetectable samples were substituted with the detection limit.

### 2.4. Data analysis

Statistical tests were conducted with SAS version 9.2 using data from reproductive females only. Scaled body mass index was calculated according to the methods described by Peig and Green (Peig and Green, 2009). Hormone concentrations were natural log transformed where necessary to meet assumptions of normality and homogeneity of variances across sampling periods (Levene's, all tests  $P > 0.05$ ). A single outlier was removed from the dataset: a measure of post-breeding testosterone ( $z = 8.3$ ). Inclusion of this datapoint does not qualitatively change any of the results. As previously reported, baseline corticosterone levels are much lower in reproductive than non-reproductive females during

the breeding season (Vitousek et al., 2010), and a high proportion of samples from reproductive females fall below the detection threshold during the breeding season (>50%). Because measurements of baseline corticosterone in reproductive females violated assumptions of normality and homogeneity, the relationship between baseline corticosterone and mate selectivity was examined separately using non-parametric statistics. Sample sizes differ slightly among tests because in a few instances we were unable to collect sufficient plasma within the allotted time to assay all physiological measures.

Mate selectivity, which was normally distributed, was modeled with a generalized linear mixed model (GLMM) that included scaled mass index and hormone measures (testosterone and stress-induced corticosterone) as fixed effects, and year and plasma sampling date as random effects. Prior to model construction it was determined that hormone measures were not correlated with each other, or with scaled mass index. Satterthwaite correction was used to estimate degrees of freedom in all GLMMs. We also analyzed these relationships substituting the magnitude of the stress response (the difference between stress-induced and baseline corticosterone) for the absolute concentration of corticosterone following the stressor. These variables were highly correlated ( $r = 0.968$ ,  $P < 0.0001$ ), and the results were not qualitatively different, so we present only the models that include stress-induced corticosterone. The relationship between mate selectivity and baseline corticosterone, which was non-normally distributed, was tested separately using a Spearman's rank correlation.

Another set of generalized linear mixed models were constructed to test the predictions that older females would promote reproduction by more strongly suppressing their corticosterone response to a stressor and maintaining lower circulating testosterone. Each model included body length (a rough proxy for age (Wikelski and Trillmich, 1997) as a fixed effect, and year and plasma sampling date as random effects. Separate models examined the relationship between body length and each hormone during the early breeding period, and during the post-breeding period.

The repeatability of hormone concentrations in reproductive females was calculated using the ANOVA-based method (Lessels and Boag, 1987; Nakagawa and Schielzeth, 2010). Repeatability was calculated twice, first using measurements from the breeding period alone, during which time repeated captures were conducted at only one of our two sampling sites (Nov 10–Dec 29;  $n = 16$  individuals). Repeatability was calculated again using samples from both the breeding and post-breeding periods (Nov 10–Jan 8;  $n = 37$  individuals). We were not able to reliably assess individual variation in baseline corticosterone because of the number of samples that fell below the detection threshold.

### 3. Results

Individual variation in stress-induced corticosterone levels (mean  $\pm$  SD:  $1.38 \pm 1.32$  ng/mL) during the early breeding period significantly predicted mate selectivity in reproductive females ( $F_{1,4.9} = 10.64$ ,  $P = 0.023$ ; Fig. 1), but circulating testosterone ( $F_{1,1.3} = 0.18$ ,  $P = 0.733$ ;  $0.84 \pm 0.55$  ng/mL) did not. Scaled mass index was not significantly associated with mate selectivity, but females with greater scaled mass showed a trend towards being more selective about potential mates ( $F_{1,9.4} = 4.22$ ,  $P = 0.068$ ;  $0.99 \pm 0.09$ ). Baseline corticosterone was also unrelated to mate selectivity ( $\rho = -0.177$ ,  $n = 17$ ,  $P = 0.496$ ;  $0.44 \pm 0.21$  ng/mL).

Stress-induced corticosterone was individually repeatable throughout the early breeding and mate selection periods ( $R = 0.37$ ,  $P = 0.037$ ), but circulating testosterone was not ( $R = -0.14$ ,  $P = 0.746$ ). Neither stress-induced corticosterone nor

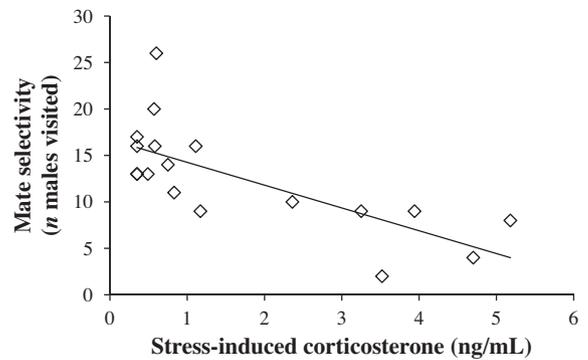


Fig. 1. Mate selectivity (the total number of unique males visited by each female during the mate choice period) was strongly predicted by individual variation in stress-induced corticosterone levels during the early breeding season in reproductive female Galápagos marine iguanas ( $n = 18$ ).

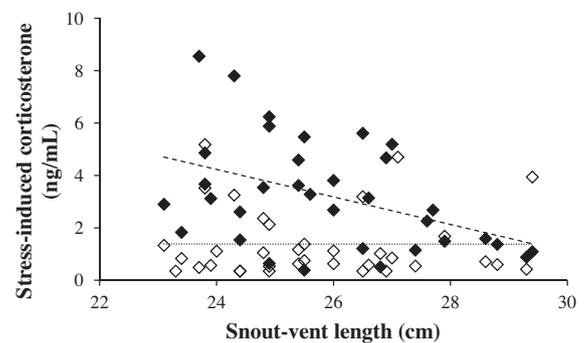


Fig. 2. Body length was not associated with stress-induced corticosterone during the early breeding season (open symbols, dotted line;  $n = 35$ ). Post-breeding and prior to egg-laying, longer (older) females secreted less corticosterone in response to stress than shorter reproductive females (filled symbols, dashed line;  $n = 34$ ).

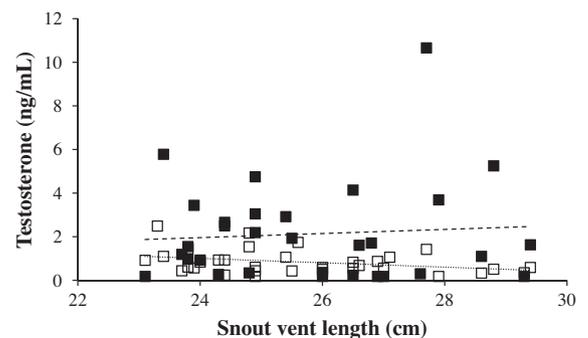


Fig. 3. During the early breeding season, longer females had lower circulating testosterone (open symbols, dotted line;  $n = 33$ ). Post-breeding and prior to egg-laying, circulating testosterone was unrelated to body length (filled symbols, dashed line;  $n = 32$ ).

testosterone were repeatable between the breeding and post-breeding periods in reproductive females (testosterone:  $R = -0.03$ ,  $P = 0.597$ ; stress-induced corticosterone:  $R = 0.05$ ,  $P = 0.337$ ).

Individual variation in stress-induced corticosterone was not associated with body length during the early breeding period ( $F_{1,32} = 0.03$ ,  $P = 0.872$ ); however, during the post-breeding period, longer females displayed weaker stress responses ( $F_{1,31.3} = 6.56$ ,  $P = 0.015$ ; Fig. 2). Longer females had lower testosterone titers during the early breeding period ( $F_{1,30} = 8.19$ ,  $P = 0.008$ ), but did not

differ in testosterone post-breeding ( $F_{1,27.9} = 0.14$ ,  $P = 0.711$ ; Fig. 3).

#### 4. Discussion

Marine iguanas are facultatively biennial reproducers. Previous analyses have found that reproductive females suppress their corticosterone stress response for the duration of the breeding period, and begin to increase the response to stressors between mate selection and egg laying (Vitousek et al., 2010). Here we find that among reproducers, individuals that show the weakest corticosterone stress response during the early breeding period, just prior to mate selection, are the most selective about potential mates. Consistent with some studies in other taxa (Angelier et al., 2009b; Cokkrem et al., 2009; but see, Ouyang et al., 2011a), stress-induced corticosterone levels were individually repeatable within life history stages (the breeding period) but not across stages (between breeding and post-breeding).

Mate choice is unusually costly in female marine iguanas, who pay direct physiological costs, as well as apparent survival costs, as a result of the energy expended assessing potential mates (Vitousek et al., 2007). Mate selectivity is also plastic and responsive to environmental conditions: reproductive females display reduced selectivity and lower mean stress-induced corticosterone levels in years when resources are scarce (Vitousek, 2009). The strong associations between individual variation in the amount of corticosterone secreted in response to a stressor and reproductive effort within and across years and reproductive classes support the prediction that the corticosterone stress response is suppressed in reproductive females in order to promote costly reproductive behaviors during the temporally constrained breeding season (Wingfield and Sapolsky, 2003) of female Galápagos marine iguanas. Experimental studies are needed to assess whether the increased corticosterone produced by individuals that respond more strongly to stressors directly influences mate selectivity – with transient increases in corticosterone reducing mate selection behaviors – or whether other physiological factors may drive this relationship. For example, in individuals capable of investing more in reproduction, the physiological stress response could be suppressed, potentially as a precaution against excess corticosterone disrupting reproduction, without circulating corticosterone mediating mate selectivity *per se*.

We predicted that individual variation in baseline corticosterone – which plays a central role in metabolic regulation – would be unrelated or positively related to mate selectivity except under conditions of chronic stress. Female iguanas in the study population do not appear to have been under chronic stress: body condition indices were similar to those observed in other years, and despite minor fluctuations in food availability (Vitousek, 2009), reproductive behavior and corticosterone levels did not come close to those observed during periods of chronic stress (Laurie, 1990; Romero and Wikelski, 2001). During the study period, individual variation in baseline corticosterone was not associated with mate selectivity. However, reproductive females maintain extremely low baseline corticosterone levels during breeding, with circulating corticosterone often falling below the detection threshold in reproductive females (but not in non-reproductive females sampled during the same period: Vitousek et al., 2010). We were therefore unable to assess the full range of inter-individual differences in baseline corticosterone in the study population, which may have prevented us from detecting a positive or negative relationship between baseline corticosterone and mate selectivity.

Circulating testosterone during the early breeding period was not predictive of mate selectivity in female marine iguanas. Data from females measured repeatedly throughout the mate selection

period indicated that testosterone titers were not individually repeatable during the breeding period, or between breeding and post-breeding. Circulating testosterone also did not covary with corticosterone. It is possible that testosterone levels during mate selection alone are predictive of mate selectivity, and that our sampling schedule precluded the detection of this pattern. However, during the mate selection period territory visitation rates remain relatively consistent within individuals while testosterone levels do not, suggesting that this hormone does not play a major role in mediating mate selectivity. Although testosterone regulates a variety of reproductive behaviors, particularly in males (Hau, 2007; Ketterson and Nolan, 1999), it is not known whether individual variation in testosterone plays a role in mediating mate choice. The implantation of exogenous testosterone in female dark-eyed juncos (*Junco hyemalis*) reduces mate selectivity and preferences, but not total attendance time, in paired mate-choice trials (McGlothlin et al., 2004). To our knowledge, it has not previously been determined whether individual variation in testosterone secretion is associated with female mate selectivity in any species.

Because older individuals have fewer opportunities for future reproduction, we predicted that older females would exhibit a weaker corticosterone response to a standardized stressor in order to facilitate the current reproductive attempt (Bókony et al., 2009; Heidinger et al., 2006; Lendvai et al., 2007; Wingfield and Sapolsky, 2003). Several previous studies have found an age-related decline in stress responsiveness in breeding birds (Heidinger et al., 2006, 2010; Angelier et al., 2007), and a greater suppression of the corticosterone stress response as the season progresses and the possibility of re-nesting decreases (Lendvai et al., 2007). Contrary to our predictions, during the early breeding period, body length (a rough proxy for age in these indeterminate growers (Wingfield and Farner, 1975) was not related to individual variation in stress-induced corticosterone in reproductive females; all reproductive females exhibited low stress-induced corticosterone levels. However, during the pre-laying period, smaller (younger) females mounted a stronger stress response than their larger counterparts.

This effect is unlikely to be due to an age-related decline in the capacity to mount a corticosterone stress response (Heidinger et al., 2008; Stein-Behrens and Sapolsky, 1992) because adult females that are not reproductively active in a given year show robust corticosterone responses regardless of body size (Vitousek et al., 2010; Vitousek, unpublished data). While mating occurs quite synchronously in marine iguanas (Rubenstein and Wikelski, 2003) we were not able to control for mating date in our analyses because not all copulations could be observed. If larger females mate earlier, and begin to increase their corticosterone response to stress before their smaller and later-mating counterparts, the observed pattern might result. Alternatively, optimal investment may be associated with age only during some phases of reproduction. The egg-laying period is exceedingly costly in marine iguanas: females not only incur significant energetic costs during nest construction, defense, and egg-laying (Laurie, 1990; Rauch, 1988; Trillmich, 1983), but also face a high risk of mortality during egg-laying, due to both cave-ins of the substrate and heavy predation by Galápagos hawks (*Buteo galapagoensis*) (Laurie, 1990). Individual variation in nest construction and defense have not been studied in detail, but based on our current findings we predict differential investment by age, with longer (older) females investing more in costly nest construction and defense than their younger conspecifics.

Testosterone was not related to body length during the post-breeding period, but during the early breeding period, longer females had lower testosterone titers. This relationship does not appear to influence mate selection itself, because circulating testosterone levels were not individually consistent, and testosterone during the early breeding period was not associated with mate

selectivity. However, temporal differences in the relationship between testosterone and body size would be predicted if testosterone plays a role in mediating other physiological or behavioral components of reproduction in which optimal investment differs by age (Hau, 2007; Ketterson and Nolan, 1999).

Individual variation in reproductive decisions, including the choice of a mate, often reflects an adaptive response to dynamic contexts (Cockburn et al., 2008; Fowler-Finn and Rodriguez, 2012; Jennions and Petrie, 1997). While the fitness consequences of mate choice can impact the direction and strength of evolution (Chaine and Lyon, 2008; West-Eberhard, 1983) the physiological mediators of mating decisions remain largely unknown. Our results provide the first evidence of an association between individual variation in circulating corticosterone – which is thought to mediate other components of reproductive investment (Wingfield and Sapolsky, 2003) – and mate selectivity in free-living organisms. Experimental work is needed to address the functional basis of this association, and the heritability and drivers of within-individual plasticity in the corticosterone stress response. Understanding the links between individual variation in circulating hormones and specific behaviors that influence fitness will help to reveal how selection may operate on suites of traits to promote adaptation to differing environmental contexts.

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