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Colonization of novel White Sands habitat is associated with changes in lizard anti-predator behaviour

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Colonization of novel habitats is often associated with differences in ecological community composition. For small diurnal animals, differences in predator diversity and abundance can lead to behavioural shifts in the novel habitat. The eastern fence lizard *Sceloporus undulatus* (Bosc and Daudin, 1801) recently colonized the gypsum dunes of White Sands, a predator-poor community relative to the predator-rich community of the surrounding Chihuahuan dark-soil habitat. We used field experiments to assess *S. undulatus* anti-predator behaviour in white-sand versus dark-soil habitats, and used laboratory assays to determine whether behavioural differences could be mediated by hormonal regulation. Overall, we found that white-sand lizards were less vigilant but more wary than their dark-soil counterparts; it took them longer to detect a simulated predator, but once detected they were more likely to retreat from their perches than dark-soil lizards. At the proximate level, differences in anti-predator behaviour could not be explained by differences in plasma hormone levels (corticosterone and testosterone); we detected elevated corticosterone for lizards in our stress treatment relative to control treatment, but found no differences between habitats in baseline or acute corticosterone levels. At the evolutionary level, we suggest that differences in anti-predator behaviour may be explained by differences across habitats in predation environment, habituation, and/or the cost of retreating. Our study implicates changes in predator community composition in mediating ecological divergence in behaviour. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **103**, 657–667.

ADDITIONAL KEYWORDS: acute stress response – corticosterone – ELISA – predation – *Sceloporus undulatus* – testosterone – White Sands.

INTRODUCTION

The invasion of novel habitats is often accompanied by shifts in ecological community structure (Wilson, 1961; Diamond, 1970; Schluter, 2000). Specifically, when a previously unoccupied habitat is colonized, species richness and abundance may differ substantially from that in the ancestral habitat (Lister, 1976; Connell & Slatyer, 1977). Changes in community composition can then have dramatic impacts on interspecific interactions and behaviour, particularly if predator, prey, or competitor species are absent or reduced in number (e.g. Hasegawa, 1994; Stone, Snell & Snell, 2003; Mateo, 2007).

One specific type of change that is likely following the invasion of a novel habitat is a change in the predation environment, which in turn may be associated with adaptive changes in behavioural responses to predators, particularly for small diurnal animals that experience high levels of predation. When predator richness and/or abundance changes, prey wariness may shift (Abramsky *et al.*, 1996; Berger *et al.*, 2007; Cooper, Hawlena & Perez-Mellado, 2009). For lizards, the focal organisms of our study, wariness can be characterized by the propensity to retreat from a perch site in response to an approaching predator. Many lizards use perches for

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*Corresponding author. E-mail: jmrobertson@uidaho.edu basking, social communication, and scanning for prey. Therefore, there are costs in retreating from a perch site. The physiological costs of retreating could be substantial for ectothermic animals that rely on basking to maintain optimal body temperature (Martín & López, 1999, 2000). In addition, retreating could also result in lost opportunities for territorial defence and courtship. The propensity to retreat will be influenced by the perceived threat (Lima & Dill, 1990; Cooper & Steele, 1997; Lima, 2009), and by prior experience (Adkins-Regan, 2005; Thaker *et al*., 2010). Thus, in a predator-rich environment there is some evidence that lizards may be more wary (i.e. retreat more), whereas in a predator-poor environment dark-soil lizards may be less wary (i.e. retreat less; Diego-Rasilla, 2003; Cooper *et al*., 2009), but the generality of this finding is unclear.

Changes in anti-predator behaviours (e.g. wariness and perch-site retreating) may ultimately be influenced by the predation environment, but at the proximate level these behavioural shifts are often mediated by hormonal regulation (Adkins-Regan, 2005; Thaker, Lima & Hews, 2009a, b). Two of the major players in hormonal regulation of squamate social behaviour are corticosterone and testosterone. Corticosterone is a glucocorticoid steroid hormone that facilitates glucose mobilization (Moore & Jessop, 2003) and is often elevated during exposure to stressors such as encounters with or attacks by predators (Thaker *et al*., 2009a, b) and in social contexts (e.g. Knapp & Moore, 1996; Smith & John-Alder, 1999). Elevation of corticosterone can affect physiological and behavioural responses, often enhancing those that promote immediate survival (e.g. increased blood glucose and locomotion), while suppressing those that do not (e.g. foraging, courtship, and territorial defence; Moore & Orchinik, 1994; Sapolsky, Romero & Munck, 2000; Adkins-Regan, 2005). The inhibitory actions of corticosterone are, in some cases, mediated by the supression of testosterone (Wingfield, Smith & Farner, 1982; Moore, Thompson & Marler, 1991; Knapp & Moore, 1997). An inverse relationship is often observed between baseline corticosterone and breeding-season testosterone levels in reptiles, particulary for lizard species with prolonged (rather than explosive) breeding seasons (Moore *et al*., 1991; Moore & Jessop, 2003).

Here, we evaluate changes in anti-predator behaviour and hormonal regulation in a lizard species that has recently invaded a novel habitat, white gypsum sand dunes. The White Sands area in south-western New Mexico (USA) is a recent geological formation (~6000 years old). The white sand dunes contrast strongly with the dark soil of the surrounding Chihuahuan desert. Multiple species, including our focal taxon, *Sceloporus undulatus* Bosc and Daudin, 1801 (eastern fence lizard; see Leache, 2009 for a discussion of taxonomy) have evolved blanched body coloration at White Sands via partial loss-of-function mutations in a single gene, presumably as an adaptation for crypsis (Rosenblum, 2006; Rosenblum *et al*., 2010). Data on tail-break frequencies also support the hypothesis that substrate matching in other *S. undulatus* populations reduces conspicuousness to predators (Gillis, 1989). In addition to the stark environmental differences in substrate colour between the white-sand and dark-soil habitats, there are striking differences in ecological community composition across this ecotone. Most notably, the white-sand habitat is characterized by lower predator richness and abundance than the dark-soil habitat (Burkett & Black, 2004; McKeever, 2009; and see Results). The dark-soil habitat contains 23 species of snakes and lizards that feed on adult lizards, whereas in the heart of the white-sand habitat there are no snakes or lizards that will consume adult *S. undulatus* (Burkett & Black, 2004). The dark-soil habitat also contains a greater *density* of both reptilian and avian predators than the white-sand habitat (see Results). Thus, lizard populations inhabiting the white-sand habitat have experienced dramatic reductions in predator diversity and abundance.

Here, we quantify differences in predation environment, anti-predator behaviour, and circulating hormones across the White Sands ecotone to determine whether white-sand lizards and dark-soil lizards exhibit differences in wariness and vigilance, and whether these behavioural differences could be explained by differences in hormonal regulation.

METHODS

We quantified and compared anti-predator behaviour and hormone levels for blanched *S. undulatus* inhabiting the heart of the dunes at White Sands National Monument (Otera County, New Mexico, USA), and for dark counterparts found in the dark soil of Jornada Long Term Ecological Research Site (Doña Ana County, New Mexico, USA). These two sites are approximately 45 km apart. Hereafter we refer to these populations as 'white-sand' and 'dark-soil', respectively.

PREDATION ENVIRONMENT

Previous research quantified the community richness of reptilian predators on lizards in white-sand and dark-soil habitats of the Chihuahuan desert (Burkett & Black, 2004). To quantify differences in predator *abundance* between habitats, and to include avian predators, we used visual encounter surveys during the peak of the *S. undulatus* activity season (May–

July 2010). We recorded the number of individual predators observed by two or three researchers while slowly walking through each habitat during the time of day that corresponds to high lizard activity at each site (07:00–13:00 h). To obtain a measure of relative abundance, we calculated the number of predators per observer hour. We used a non-parametric Mann–Whitney *U*-test to test for population differences in predator abundance.

ANTI-PREDATOR BEHAVIOUR

We conducted anti-predator response trials to determine whether recently colonized white-sand lizards respond differently to predator encounters in wariness and vigilance than their dark-soil counterparts. All trials were conducted in the breeding season (between 4 June and 10 July 2009), and between 10:00 and 13:00 h (e.g. John-Alder, M. Gründler, K. Hoversten, J. Robertson, 2009; pers. observ.). We located lizards with binoculars by slowly walking through suitable habitat at each field site. We only included lizards that had been identified at a sufficient distance (> 200 m) so that they were not alerted to our presence (i.e. lizards did not re-orient to the simulated predator before the start of the trial). We studied lizards perched on *Yucca elata* (soaptree yucca) because *S. undulatus* in this region spend most of their time in this microhabitat (Hager, M. Gründler, K. Hoversten, J. Robertson, 2001; pers. observ.), and *Y. elata* is the primary shared microhabitat feature between the two habitats. White-sand and dark-soil habitats do differ somewhat in overall habitat structure, with white-sand habitat generally having sparser vegetation. Although structural differences could cause differences in the visibility of approaching threats, our results are not influenced by any differences in habitat complexity affecting the line of sight: we only tested lizards on elevated perches, and maintained clear line-of-sight conditions for the duration of each trial to ensure that the lizard's view of the simulated predator was never obstructed. We excluded lizards that were actively foraging or engaged in conspecific interactions (either courtship or territoriality). Because lizards easily evaded capture after retreating, we did not record the sex of individuals tested; however, we restricted trials to adult lizards.

We assessed anti-predator behaviour by introducing each focal lizard to an approaching human predator (M.G.). The use of a human predator is an established and standard method for lizards: previous studies have demonstrated that lizards respond to humans as if they were predators, in a manner consistent with predictions from optimal escape theory (see Cooper *et al*., 2009 for a review). Furthermore, there are advantages to using human predators that outweigh the costs of using natural predators, such as the ability to traverse uneven terrain, consistency in approach speed, and reduced threat of accidental harm to study individuals (Cooper *et al*., 2009). The researcher that simulated the predator wore the same clothing (drab-coloured shirt and trousers, hat, and sunglasses) and footwear for all trials. Before the start of each new day of trials, we timed the pacing of the 'predator' to ensure the consistency in approach rate between trials and days (Table S1; Cooper, 1997). We approached all focal lizards directly from behind, at a medium pace, and with a deliberate shuffle. We continued to approach the focal lizard until the lizard retreated or until the human predator was within inches of the focal animal, at which point the trial ended.

We measured three main response variables. First, the 'alert initiation distance' (AID) was the distance at which the focal lizard moved any part of its body to position itself with reference to the approaching human predator (reflecting vigilance). Second, the 'flight initiation distance' (FID) was the distance at which the focal lizard *started* to flee (reflecting wariness). As the human predator approached the focal lizard, a small stone marker was inconspicuously dropped when the lizard moved (AID), and also at the point when it fled (FID). After trial completion, the distances between each marker stone and the lizard's original position were measured with a 50-m measurement tape. Third, 'retreat' (reflecting wariness) was a binomial response variable that described whether the lizard fled out of sight (e.g. into the dense mat of yucca leaves at the base of the yucca stalk) or not. Note that 'flight' and 'retreat' describe different behaviors, and not all lizards that began to flee (e.g. ran any distance on the yucca stalk) retreated fully. We used non-parametric Mann–Whitney *U*-tests to determine population differences in AID and FID. We used contingency analyses, tested for significance with chi-square tests, to determine population-level differences in whether lizards retreated or not. All statistical analyses were performed in the statistical software package JMP 8.0 (SAS, 2007).

HORMONAL MEASURES

For hormone analysis we sampled blood from male *S. undulatus* specimens from both white-sand and dark-soil habitats between 7 June and 24 June 2009. In addition to blood sampling, we also measured body condition for all lizards (residuals from a regression of body mass onto snout–vent length) because body condition can contribute to a variation in corticosterone levels (e.g. in *Sceloporus occidentalis*; Dunlap & Wingfield, 1995). We followed established field-sampling and hormonal analyses for *S. undulatus*, as stress response and seasonal variation in circulating hormone levels have been well studied in other parts of the *S. undulatus* range (McKinney & Marion, 1985; Klukowski & Nelson, 1998; Smith & John-Alder, 1999; Thaker *et al*., 2010).

All blood samples were collected in the middle of the breeding season to limit seasonal variation in hormone levels (Wilson & Wingfield, 1994; Knapp *et al*., 2003; John-Alder *et al*., 2009). Upon capture by noose, we placed individuals into one of two treatment groups: control and acute-handling stress. The control group was used to measure baseline plasma corticosterone and testosterone levels. Because plasma corticosterone levels typically rise within 4 min of a stressful event (Wingfield *et al*., 1982; Wada, Hahn & Breuner, 2007), and vary with time of day (Adkins-Regan, 2005), we collected all blood samples between 08:00 and 11:00 h, and within 4 min of capture (mean ± SD = 3 min 9 s ± 1.2 s). For the acute-handling stress group, we placed each individual lizard in a separate small reptile terrarium (14.5 × 10 × 10 cm) that lacked substrate, and collected blood after 30 min (mean ± SD = 32 min 55 s ± 1.8 s). We determined that keeping lizards in captivity without substrate, but in the shade, and with adequate ventilation, sufficiently stressed the lizards without causing serious physiological decompensation. Signs of physiological decompensation include non-responsiveness to touch, failure to move away from stimulus, and inability to ‘right’ if turned over. None of our lizards exhibited any of these signs. We did not include lizards from the anti-predator trial for hormonal analyses because white-sand lizards easily evaded capture after retreating. We collected blood by rupturing the retro-orbital sinus using heparinized microcapillary tubes (e.g. Moore *et al*., 1991), and then released all individuals at their sites of capture. Blood samples were kept on ice (1–3 h) until centrifuged to separate the plasma from the cellular fraction. We aliquoted plasma into microcentrifuge tubes and stored all plasma samples at −20 °C in the field. We stored samples in the laboratory at −80 °C prior to assay.

Plasma concentrations of corticosterone and testosterone were determined by enzyme immunoassay (Assay Designs EIA kits, Corticosterone 901–097, Testosterone 901–065, Enzo Life Sciences International, Inc., Plymouth Meeting, PA). Prior to running the samples, we optimized the assay using three dilutions according to the method described by Wada *et al*. (2007). In brief, raw plasma was stripped of hormone using activated charcoal (Norit SA 2, Aros Organics CAS- 7440-440, Norit Americas Inc., Marshall TX) and subsequently spiked with 500 pg mL^{-1} of either corticosterone or testosterone (Assay Designs). For optimizations we created a plasma pool by combining samples from the white-sand and dark-soil populations. The EIA protocol was carried out for three plasma dilutions (1 : 20, 1 : 40, and 1 : 80) and three steroid displacement buffer (SDB) concentrations (1, 2, and 3%), with the kit-supplied assay buffer. We found the optimal combination to be 1 : 80 plasma and 2% SDB for both hormones and for each population, and subsequently used this combination for each EIA. The corticosterone and testosterone EIAs were performed according to the protocol provided by the kit’s manufacturer. The samples were assayed in triplicate and randomized across each plate. We determined the optical density of each well at 415 nm using a BIORAD 680 Microplate Reader (Bio-Rad, Hercules, CA). The concentration of plasma hormone was calculated by interpolation from a standard curve, performed in duplicate on each plate. Because some corticosterone samples had a wide variation in concentration among replicates with 1 : 80 ratio, we re-assayed them at more dilute plasma concentrations to fall within the optimum assay range of 100–500 pg mL^{-1}. The repeated samples were assayed with dilutions varying from 1 : 640 to 1 : 2560. We re-assayed seven lizard samples in the stress treatment (four from the dark-soil; three from white-sand). Mean intra-assay coefficients of variation were not calculated because we only had duplicate values of hormone standards. Inter-assay coefficients of variation were 33.2% for corticosterone and 27.8% for testosterone. We do not draw conclusions from individual values, but rather detect relative differences between groups. Samples from each treatment group were randomized within and among assay plates, and we were able to detect significant differences among treatment groups (see Results).

We used standard least-squares regression to test for the effect of population, treatment, body condition, and their interactions on hormone level. We log-transformed data prior to analyses for normality. From each model, the *F* ratio and associated degrees of freedom were used to generate a *P* value to test for significance of each effect. Because body condition was not signficant in either corticosterone and testosterone models (see Results), it was excluded from further analyses. We used post-hoc one-way ANOVAs to test for specific differences in corticosterone and testosterone across treatment groups and between habitats.

RESULTS

PREDATION ENVIRONMENT

Predator abundance was higher in the dark-soil habitat than in white-sand (Mann–Whitney *U*-test normal approximation $z = -4.6$, $P < 0.00001$; Fig. 1).

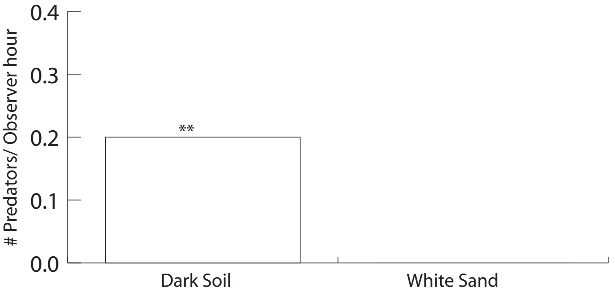

Figure 1. Difference in avian and reptilian predator abundance between dark-soil and white-sand habitats. The number of predators per observer hour was higher in the dark habitat. Means and standard errors are shown. *$P < 0.05$; **$P < 0.0001$.

In dark-soil, we observed 30 individual predators in 148.20 observer hours, but observed zero predators at white sand in 131.80 observer hours. The predators commonly observed in the dark-soil habitat included *Gambelia wislizenii* (the long-nosed leopard lizard), *Crotaphytus collaris* (the collared lizard), *Crotalus viridis* (the western rattlesnake), *Masticophus flagellum* (the coachwhip snake), *Pituophis catenifer* (the gopher snake), and the birds *Geococcyx californianus* (the greater roadrunner), and *Buteo swainsoni* (Swainson's hawk). Although roadrunners, loggerhead shrikes (*Lanius ludovicianus*) and hawks occur at the white-sand site, none were observed during our surveys, underscoring their relatively low abundance.

ANTI-PREDATOR BEHAVIOUR

We quantified anti-predator behaviour for 42 adults from each population for a total of 84 trials. Dark-soil lizards exhibited greater AIDs (7.0 m ± 0.9 m) than white-sand lizards (5.2 m ± 0.9 m; Mann–Whitney *U*-test, $z = -2.37$, $P = 0.009$; Fig. 2). In other words, dark-soil lizards were alert to predators sooner than white-sand lizards. The FID did not differ between white-sand (2.5 m ± 0.5 m) and dark-soil populations (2.4 m ± 0.4 m; Mann–Whitney *U*-test, $z = -0.51$, $P = 0.3$). The likelihood of retreat differed significantly between populations: white-sand lizards retreated in 76% of trials, whereas only 12% of dark-soil individuals retreated ($\chi^2_1 = 31.9$, $P < 0.0001$, Fig. 2), indicating that white-sand lizards were more wary than dark-soil lizards.

HORMONAL MEASURES

We measured corticosterone and testosterone from ten control and 12 acute-handling-stress adult males from each population for a total of 44 individual blood samples. Body condition was not significant in a full least-squares regression model for either corticosterone (parameter estimate = –0.13, $P = 0.531$) or testosterone (parameter estimate = 0.030, $P = 0.784$). Therefore, body condition was removed as a covariate in subsequent analyses.

For corticosterone, the full least-squares regression model (which included a population effect, treatment effect, and population by treatment interaction) detected a significant effect of treatment only (ANOVA, $F_{2,41} = 9.89$, $P = 0.0003$; Fig. 3A; Table 1). That is, there was no difference between populations in corticosterone for either the control or stress treatment, but the stress-treatment group had significantly higher levels of corticosterone relative to the control group. High interassay variation (27–33%) could have reduced our power to detect subtle population-level difference, but the difference between control and stress animals in corticosterone was clear, despite this variation.

For testosterone, the full least-squares regression model identified significant effects of population, treatment, and their interaction (ANOVA, $F_{4,27} = 9.653$, $P < 0.0001$; Fig. 3B; Table 1). Post-hoc one-way ANOVAs detected significant differences between habitats in testosterone for both stress ($F_{1,22} = 5.771$, $P = 0.025$) and control ($F_{1,19} = 60.78$, $P < 0.0001$; Fig. 3B) treatment, with significantly higher levels of testosterone in the dark-soil lizards relative to the white-sand lizards. Levels of testosterone did not differ between control and stress groups in the white-sand population (Student's *t*-test, $t_{1,20} = 0.0004$, $P = 0.984$), but were higher for the control group relative to the stress-treatment group sampled in the dark-soil population (Student's *t*-test, $t_{1,20} = 5.07$, $P < 0.0001$).

DISCUSSION

White-sand lizards exhibit rapid adaptation to a novel habitat through a suite of phenotypic and behavioural traits. We have shown previously that white-sand *S. undulatus* differ from their dark-soil counterparts in dorsal coloration (Rosenblum, 2006), social signal coloration (Robertson & Rosenblum, 2009), body size and shape (Rosenblum & Harmon, 2010), and social behaviour (Robertson & Rosenblum, 2010). Here, we demonstrate differences between white-sand and dark-soil lizards in anti-predator behaviour. We report that white-sand lizards were less vigilant but more wary than dark-soil lizards. Below, we discuss both ultimate (e.g. changes in predator community) and proximate (e.g. changes in hormonal regulation) mechanisms that are likely to explain our results.

ANTI-PREDATOR BEHAVIOUR

Overall, white-sand lizards were more wary but less vigilant than dark-soil lizards. White-sand lizards

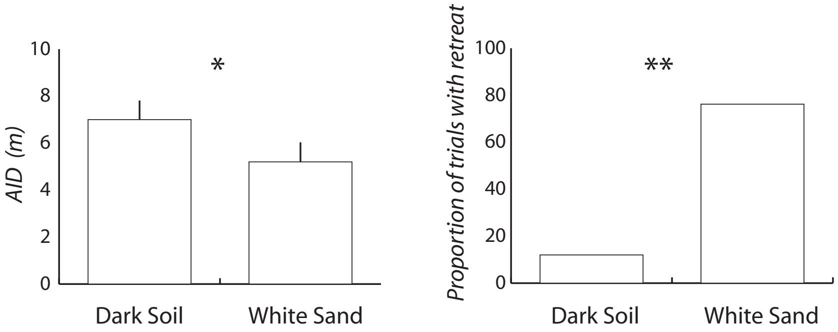

Figure 2. Anti-predator behaviour trials demonstrated that white-sand *Sceloporus undulatus* were less vigilant but more wary than dark-soil conspecifics. White-sand lizards exhibited shorter alert initiation distances (AIDs) compared with dark-soil lizards. Means and standard errors are shown for AID. White-sand lizards retreated from perch sites in a higher proportion of trials than dark-soil lizards. *P < 0.05; **P < 0.0001.

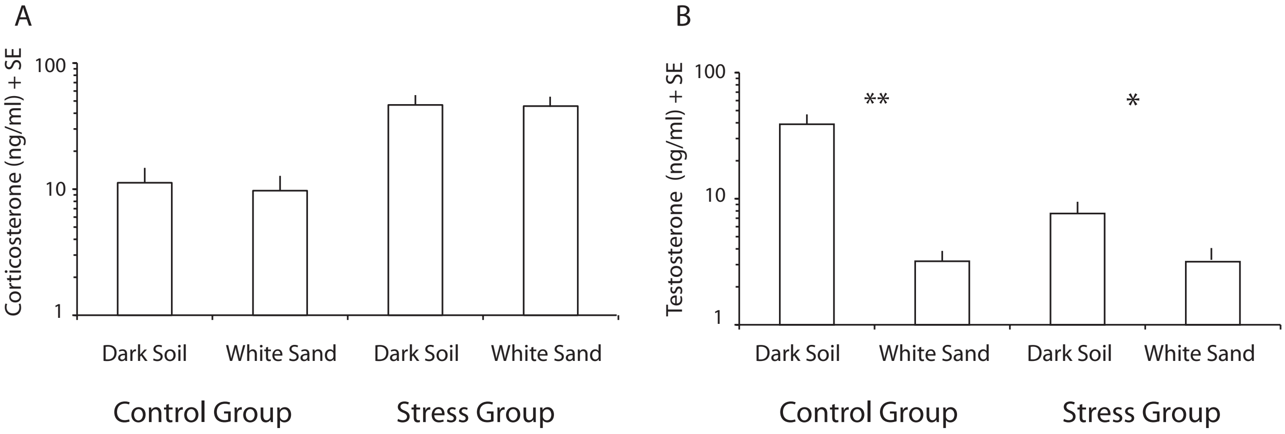

Figure 3. Hormonal response in control and stress-treatment groups for white-sand and dark-soil *Sceloporus undulatus* males. A, Mean corticosterone was higher in the stress-treatment groups compared with control groups, but did not differ between habitats. B, Mean testosterone was higher for dark-soil lizards than white-sand lizards in both control and stress groups. Mean testosterone was also higher for dark-soil lizards in the control relative to the stress-treatment group. Means and standard errors are shown in both panels. *P < 0.05; **P < 0.0001.

Table 1. Standard Least Squares regression for predicting the effect of population, treatment group, and their interaction on corticosterone and testosterone in dark-soil and white-sand *S. undulatus*. F ratio and associated degrees of freedom (df) are used to generate a p-value to test for effect significance. DS = dark soil. C = Control group

	Corticosterone				Testosterone			
Source	Estimate	df	F ratio	p-value	Estimate	df	F ratio	p-value
Population DS	0.110	1,28	1.47	0.237	0.258	1,23	4.232	0.0002
Treatment C	–0.405	1,28	19.90	0.001	0.126	1,23	13.384	0.0143
Population DS *Treatment C	0.071	1,28	0.633	0.434	0.145	1,23	4.232	0.0056

were more likely to retreat than dark-soil lizards (i.e. they were more wary; Fig. 2). However, white-sand lizards exhibited shorter AIDs than their dark-soil counterparts (i.e. less vigilant, Fig. 2). Retreat and vigilance are different forms of anti-predator behaviour. Differences across populations in vigilance or wariness, of course, can be influenced by stochastic processes like genetic drift. However, we propose that our observations can largely be explained by differences in predator environment and differences in lizard physiology across habitats.

Habituation is one likely explanation for differences in retreat behaviour across habitats. Because not only predators, but also other non-predatory birds and reptiles, are more diverse and more numerous in dark-soil habitat, dark-soil lizards may be habituated to high levels of surrounding activity (e.g. Romero & Wikelski, 2002). Prey, such as small lizards, will alter their behaviour in response to a perceived threat of predation (Lima & Dill, 1990). Therefore, dark-soil lizards may retreat less often because they have learned to distinguish between threatening and non-threatening predators. For example, in an eastern population of *S. undulatus*, lizards distinguished between greater and lesser threats, and with repeated exposure to these simulated predators, lizards were more bold, retreating for shorter durations (Thaker *et al*., 2010). Because white-sand lizards inhabit a reduced predation environment, the lack of habituation could explain their relative wariness (e.g. Cooper *et al*., 2009).

An alternative and non-mutually exclusive explanation for the observed differences in retreat behaviour is the differential cost of retreat between habitats. Fleeing from an approaching predator can incur a cost of lost opportunities for thermoregulation, feeding, and mating (Adams *et al*., 2006). The actual cost of retreat may be higher for dark-soil lizards. In dark-soil habitat, there is a higher diversity and abundance, not only of avian predators, but also of ground and shrub-dwelling predators (i.e. larger predatory lizards and snakes). Because these terrestrial predators are absent in the core of the white-sand habitat, white-sand lizards may suffer no additional predation risk from ground-dwelling predators by retreating to the ground. Other factors could decrease the costs (or increase the benefits) of retreating for white-sand lizards. For example, physiological differences in thermoregulation between habitats could influence the cost-benefit ratio of retreat. However, thermoregulatory factors are unlikely to explain our results because the predicted costs are opposite to the observed patterns of retreat. Specifically, white-sand lizards should suffer an increased thermoregulatory cost of retreat relative to their dark-soil counterparts because darker lizards can increase their temperatures more quickly by basking, than can lighter lizards (Luke, 1989).

Although white-sand lizards were more wary than dark-soil lizards (with a higher likelihood of retreat), they were less vigilant (shorter AIDs). There are two alternative hypotheses for the observed differences in vigilance. First, dark-soil lizards may be more vigilant because they inhabit a high predation environment, where it is important to assess the relative threat of potential predators (Thaker *et al*., 2010). In contrast, predators in white-sand habitat are so uncommon, vigilance may not be under as strong selection in this habitat. Alternatively, the shorter AIDs in white-sand lizards may result from evolved differences in the visual system of white-sand *S. undulatus*. The white-sand habitat is an extremely bright environment, exagerated by the reflectance of the sun off of the pure white gypsum. Evidence suggests reduced visual sensitivity in white-sand lizards relative to dark-soil lizards (Nava, 2009). Therefore, our observation of reduced AIDs in white-sand lizards may not be the result of specific changes in vigilance, but simply a limit on visual acuity and movement perception in white-sand *S. undulatus*.

Hormonal regulation of behaviour

We predicted that differences in anti-predator behaviour would be mediated by hormonal responses to stress, as measured by blood plasma levels of corticosterone. Higher average 'baseline' levels might also be expected in populations with higher rates of predator encounter. However, we did not detect differences in baseline or stress corticosterone levels between white-sand and dark-soil lizards (although subtle differences might not have been detectable, given the inter-assay variation observed in our samples). We did demonstrate that lizards in both habitats responded rapidly to acute handling stress, with an average 4.4-fold increase in corticosterone (compared with respective control lizards; Fig. 3A), indicating that our corticosterone assay effectively measured a stress response in these lizards. Thus, our results suggest that differences in plasma corticosterone levels alone are unlikely to explain population differences in anti-predator behaviour.

A lack of association between anti-predator behaviour and corticosterone response to stress has also been observed in other squamate taxa (Adkins-Regan, 2005). For example, morph-specific differences in anti-predator behaviour of the ornate tree lizard *Urosaurus ornatus* were not associated with differences in cortiocosterone responses to predator exposure (Thaker, Lima & Hews, 2009a). Other hormonal mechanisms that may account for behavioural differences include the differential expression of corticosterone-releasing hormone (Oswald, 2010), a reduced number of glucorticoid receptors, or differences in plasma-binding globulin capacities or affinities (e.g. Breuner *et al*., 2003; Charlier *et al*., 2009). Because our EIA measured total levels of hormone (both bound and free), we could not assess whether free corticosterone concentrations differed between white-sand and dark-soil lizards. Only free corticosterone (not bound to a glucocorticoid-binding globulin) is thought to exert a physiological effect on the organism (e.g. Breuner *et al*., 2003). Therefore, although our lizard populations did not differ in total corticosterone, differences in behaviour could nonetheless be mediated by corticosterone if the populations differ in binding globulin capacity, which has been shown for ornate tree-lizard morphs (Jennings *et al*., 2000). Studies that assess glucocorticoid receptor abundance, expression, and plasma-binding globulins would more fully resolve whether corticosterone mediates behavioural differences between populations. Finally, the captivity container used in the stress-treatment group could elicit a stronger stress response than our approaching human-predator treatment. If so, corticosterone levels may have been maximal for lizards in the stress treatment for both populations, and may not reflect what might be elicited by a predator encounter. Additional experiments designed to detect subtle changes in corticosterone regulation in response to more natural stress conditions could be used to more fully characterize the role of corticosterone in anti-predator behaviour.

In contrast to the lack of population differences in corticosterone, dark-soil lizards consistently exhibited higher levels of testosterone than white-sand lizards (i.e. in both control and stress-treatment groups). Although we did not hypothesize a direct role for testosterone in *S. undulatus* anti-predator behaviour, from the functional perspective, testosterone is an androgen steroid hormone associated with aggression (Huyghe *et al*., 2009), territory defence, and courtship in many reptiles (Moore & Lindzey, 1992; Adkins-Regan, 2005). For example, in mountain spiny lizards, *Sceloporus jarrovii* (Moore, 1987), ornate tree lizards, *Urosaurus ornatus* (Kabelik, Weiss & Moore, 2008), side-blotched lizards, *Uta stansburiana* (Denardo & Licht, 1993), and brown anole lizards, *Anolis sagreii* (Tokarz *et al*., 2002), experimental manipulations of testosterone revealed a positive association between testosterone and aggressive displays in male–male interactions. Studies of non-manipulated males, however, have often failed to find an association between individual levels of aggressive behaviour and circulating testosterone (e.g. Knapp & Moore, 1996; Hews & Moore, 1997; Baird & Hews, 2007), suggesting that other mechanisms also commonly mediate aggresive behaviour in nature. Although our study did not measure associations of individual territorial behaviours with plasma testosterone levels, a population-level study of male territorial behaviour found no differences in aggression between white-sand and dark-soil males (Robertson & Rosenblum, 2010). Thus, the observed differences in testosterone are not associated with any clear differences in aggression between populations.

An alternative and more likely possibility to explain the observed population differences in testosterone is that dark-soil and white-sand populations were asynchronous in the timing of peak breeding activity. Testosterone varies seasonally, with peak levels occurring early in the breeding season and reduced levels in the middle of the summer (e.g. McKinney & Marion, 1985; Klukowski & Nelson, 1998; John-Alder *et al*., 2009). Our sampling occurred during the breeding season for both populations, and in fact blood collection for hormonal assays occurred within the same week. Despite their geographic proximity, there are abiotic differences between white-sand and dark-soil habitat that could lead to differences in the timing of peak reproductive activity (e.g. mean air temperature; Hager, 2001). In fact, we observed that the breeding season in 2009 ended earlier for the white-sand population compared with the dark-soil population (pers. observ. M. Gründler, K. Hoversten, J. Robertson, E. B. Rosenblum). Additional study is needed to determine whether subtle differences in the peak timing of reproductive activity across habitats could explain the observed population differences in testosterone levels.

A final observation for testosterone was that, for the dark-soil population only, mean testosterone levels were significantly lower following a 30-min handling stress treatment compared with the control group. One mechanistic explanation for the inverse patterns observed in testosterone and corticosterone involves plasma-binding globulins. Whereas plasma-binding globulins are poorly understood in reptiles, in ornate tree lizards, *Urosaurus ornatus*, a single binding globulin, the androgen glucocorticoid-binding globulin (AGBG), has a high affinity for both corticosterone and androgens (Jennings *et al*., 2000). The capacity of AGBG can vary between individual lizards (e.g. male morphs in ornate tree lizards; Jennings *et al*., 2000) and among populations (e.g. white crowned sparrows; Breuner *et al*., 2003). Assessing these steroid hormone binding globulins in our two lizard populations would be especially informative.

Our research demonstrates differences in anti-predator behaviour between recently diverged blanched lizards at White Sands and dark lizards in the surrounding Chihuahuan desert. In general, white-sand lizards were less vigilant and more wary than dark-soil conspecifics. The observed changes in anti-predator behaviour are probably the result of shifts in predator richness and abundance in the novel white-sand habitat, but could not be directly linked to population differences in hormonal cues. This research provides an empirical example of how ecological changes can influence animal behaviour, and contributes to a growing body of work demonstrating that suites of morphological and behavioural traits can exhibit rapid and correlated change upon invasion of novel habitats.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Pacing times (s) and daily average for the simulated (human) predator to walk 15.24 m. All pacing trials (T1–T8) were performed before the start of predator trials for that day. The average pacing time to walk 15.24 m, across all dates and trials, was 8.82 s. We found no obvious trends in pacing time across the study trials, indicating that our results were not biased as a result of differences in predator approach speed between trials.