Ancestry trumps experience: Transgenerational but not early life stress affects the adult physiological stress response

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ABSTRACT

Exposure to stressors can affect an organism's physiology and behavior as well as that of its descendants (e.g. through maternal effects, epigenetics, and/or selection). We examined the relative influence of early life vs. transgenerational stress exposure on adult stress physiology in a species that has populations with and without ancestral exposure to an invasive predator. We raised offspring of eastern fence lizards (*Sceloporus undulatus*) from sites historically invaded (high stress) or uninvaded (low stress) by predatory fire ants (*Solenopsis invicta*) and determined how this different transgenerational exposure to stress interacted with the effects of early life stress exposure to influence the physiological stress response in adulthood. Offspring from these high- and low-stress populations were exposed weekly to either sub-lethal attack by fire ants (an ecologically relevant stressor), topical treatment with a physiologically-appropriate dose of the stress-relevant hormone, corticosterone (CORT), or a control treatment from 2 to 43 weeks of age. Several months after treatments ended, we quantified plasma CORT concentrations at baseline and following restraint, exposure to fire ants, and adrenocorticotropic hormone (ACTH) injection. Exposure to fire ants or CORT during early life did not affect lizard stress physiology in adulthood. However, offspring of lizards from populations that had experienced multiple generations of fire ant-invasion exhibited more robust adult CORT responses to restraint and ACTH-injection compared to offspring from uninvaded populations. Together, these results indicate that transgenerational stress history may be at least as important, if not more important, than early life stress in affecting adult physiological stress responses.

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1. Introduction

An organism's environment in early life, including the availability of nutrients, maternal behavior, and presence of predators and competitors, can have important consequences for physiology, morphology, and behavior that persist into or manifest during adulthood (Champagne and Meaney, 2001; Kasumovic, 2013; Relyea, 2001). For example, increased exposure to stressors during early life (i.e. prior to adulthood) can program physiology and behavior (Dalmaz et al., 2015; de Kloet et al., 2005; Meaney et al., 1994), and lead to elevated aggression, anxiety, and depressive behaviors in adult rodents and primates (reviewed in Heim and Nemeroff, 2001; McCormick and Green, 2013; Veenema, 2009). These effects of early life stress may be

modulated by an individual's genotype (de Kloet et al., 2005; Lightman, 2008; Oitzl et al., 2010). Additionally, stress experienced by an individual can affect stress physiology of its offspring (Harris and Seckl, 2011; Storm and Lima, 2010; Yehuda et al., 2000) and grandchildren (Bertram et al., 2008; Harris and Seckl, 2011; Matthews and Phillips, 2012, 2010). In fact, transgenerational stress programming may influence how individuals respond to early life stress (e.g. Sheriff and Love, 2013). Evidence of these changes is typically observed over one or two generations (i.e. in the F1 or F2 generation; Matthews and Phillips, 2012). However, exposure to stressors over many more generations may be necessary to elicit such changes if they are the product of natural selection. Mechanisms underlying these changes could thus include plasticity, epigenetics, maternal effects, and/or natural selection (Anacker et al., 2014; Jablonka and Raz, 2009; Jenkins et al., 2014; Love and Williams, 2008).

When persistently exposed to stressors either within a lifetime (e.g. during early life) or across generations, an organism's physiological stress response may change to balance associated costs and benefits (Matthews, 2002; Meaney et al., 1994; Oitzl et al., 2010). This could take the form of up- or down-regulation of the hypothalamic-

pituitary-adrenal (HPA) axis, resulting in increased or decreased baseline or post-stress concentrations of stress hormones (e.g. corticosterone, CORT; adrenocorticotrophic hormone, ACTH; corticotrophin-releasing hormone, CRH; e.g. Carpenter et al., 2007; Heim et al., 2000; Ladd et al., 1996; Plotsky and Meaney, 1993). Suppression of the stress response could reflect habituation to a specific stressor (Grissom and Bhatnagar, 2009; Romero et al., 2009; Romero and Reed, 2005) or general down-regulation of the response (Romero, 2004). This suppression may protect the organism from stress-related costs (e.g. diversion of energy away from non-immediately-critical functions such as growth, reproduction, and immune function; Chrousos and Gold, 1992; Greenberg and Wingfield, 1987; Martin, 2009) but could reduce benefits associated with heightened stress reactivity (e.g. supporting behavioral and metabolic responses to stressors; Sapolsky, 2000; Thaker et al., 2009). Alternatively, up-regulation of the stress response may allow an organism to more effectively mount responses to current threats (Romero, 2004) while potentially incurring longer-term costs of elevated CORT (Korte et al., 2005; Romero et al., 2009; Sapolsky et al., 2000). Both up- and down-regulations of the adult stress response has been observed in response to early life stress (Ariza Traslaviña et al., 2014; Carpenter et al., 2007; Caruso et al., 2014; Spencer et al., 2009) and in some cases no change is observed (e.g. adult baseline CORT; Mirescu et al., 2004; Plotsky and Meaney, 1993). The effects of early life stress are relatively well studied in humans and rodents (McCormick and Green, 2013; Veenema, 2009). Expanding this to other organisms with documented differences in ancestral stressor exposure will inform our understanding of the evolutionary pressures leading to the consequences of both early life and transgenerational stress.

A variety of environmental perturbations are known to induce the physiological stress response, including habitat loss (Homan et al., 2003; Suorsa et al., 2004), urbanization (French et al., 2008), and the introduction of invasive species (Berger et al., 2007; Graham et al., 2012). We took advantage of naturally occurring lizard populations experiencing high or low stress as a result of multiple generations (>31 lizard generations) of exposure to predatory invasive fire ants or no history of exposure, respectively (Graham et al., 2012). Red imported fire ants (*Solenopsis invicta*) are invasive predators of eastern fence lizards (*Sceloporus undulatus*), and these species utilize similar habitat where their ranges overlap (Langkilde, 2009a, 2009b). Fire ants frequently attack fence lizards in nature (Freidenfelds et al., 2012). These encounters induce anti-predator behavior (Langkilde, 2009b) and trigger the release of CORT in lizards (Graham et al., in press). Lizards in areas invaded by fire ants have higher baseline concentrations of CORT than do those from uninvaded sites (Graham et al., 2012). We manipulated early life stress of offspring of lizards from these high- and low-stress populations and measured aspects of their stress physiology in adulthood. This study was not designed to identify the specific mechanisms of any observed change, but allowed us to experimentally investigate whether adult HPA activity is affected by early life and transgenerational exposure to stressors and whether these stressor histories interact. This is one of the first studies to our knowledge that considers the effect of stress exposure across multiple generations on adult physiology, and its interaction with early life stress.

2. Methods

2.1. Study sites and animal collection

During April and May 2012, we captured gravid female eastern fence lizards ($n = 86$) from 6 sites across the southeastern United States: (1) Blackwater River State Forest, Santa Rosa County, Florida; (2) Geneva State Forest, Geneva County, Alabama; (3) Conecuh National Forest, Covington County, Alabama; (4) St Francis National Forest, Lee County, Arkansas; (5) Edgar Evins State Park, DeKalb County, Tennessee; and (6) Standing Stone State Park, Overton County, Tennessee. All sites have similar habitat (Langkilde, 2009b, unpubl. data) but differ in fire

ant invasion history (Callcott and Collins, 1996): Sites 1, 2, and 3 were first invaded by fire ants 57 to 75 years ago ("invaded;" 31 to 42 generations, based on Parker, 1994), while sites 4, 5, and 6 have no previous history of fire ant invasion ("uninvaded").

All procedures detailed below adhere to the Guidelines for the Use of Animals in Research and the Institutional Guidelines of Penn State University (IACUC #35780), and animal collection was permitted by the respective states.

2.2. Animal husbandry

Gravid lizards were housed in pairs in plastic enclosures ($56 \times 40 \times 30$ cm, L $\times$ W $\times$ H) furnished with a shelter for refuge and basking, a water bowl, and moist sand for nesting. Overhead lights were set to a 12:12 h light:dark schedule (light: 0800–2000 h), and a 60-W incandescent light bulb was placed at one end of the enclosure to provide heat for 6 h each day to allow lizards to thermoregulate. Lizards were fed crickets (*Acheta domestica*) dusted with calcium and vitamin supplements every second day, and water was available ad libitum.

We checked enclosures at least twice daily for eggs and immediately placed clutches in plastic containers ($11 \times 11 \times 7.5$ cm) filled with moist vermiculite (-200 kPa), covered with plastic wrap, and sealed with a rubber band (Langkilde and Freidenfelds, 2010). We placed containers in an incubator (29 °C $\pm$ 1 °C) until eggs hatched (approximately 45 days), rotating the containers every other day to avoid any within-incubator effects of position. We checked incubators twice daily for hatchlings.

We toe-clipped hatchlings for unique identification and housed them in groups of six based on age. Each enclosure contained two lizards from each of the three treatments (described under Treatments section) and no more than two lizards from each clutch, each from different treatments. Lizards from fire ant-invaded sites never shared enclosures with those from uninvaded sites. Hatchlings were housed under similar conditions as gravid females but without sand; the floor of each enclosure was instead lined with paper towel. At 42 weeks of age, we measured all lizards for mass and snout-vent length (SVL) and subsequently quantified aspects of their HPA activity. All data are thus from measures of these offspring and not of the parental generation.

2.3. Treatments

To determine the effects of exposure to CORT or an ecologically-relevant stressor during early life, hatchlings were assigned to one of 3 treatments using a split-clutch design. Starting at 2 weeks of age, lizards were exposed to topical application of CORT (CORT), fire ants (FA), or a handling and oil-vehicle control (Ctl) once a week for 42 weeks. This regimen was selected to be ecologically-relevant, while avoiding potentially lethal effects of frequent exposure to fire ant venom (Freidenfelds et al., 2012). To ensure all lizards received the same handling and topical application, each week all lizards were individually placed in a sand-lined arena (with or without fire ants; 9×22 cm, R $\times$ H) for 30 s, after which 3 μl sesame oil (with or without CORT) was applied to their backs with a pipette. Lizards were returned to their home enclosure after treatment.

Lizards in the fire ant treatment ($n = 100$) were placed inside the testing arena with 15–20 fire ants. Ants were allowed to encounter and sting the lizard, as they do in nature. A trial ended 30 s after the first ant contacted the lizard, and any attacking fire ants were removed from the lizard. This provided a non-lethal exposure that induces CORT elevation (Graham et al., in press). They then had sesame oil applied to their back.

Lizards in the CORT treatment ($n = 95$) topically received CORT (≥92%, Sigma C2505, Saint Louis MO) dissolved in commercial sesame oil, after being removed from the sand-lined testing arena. The oil and hormone were quickly absorbed due to the lipophilic nature of lizard skin (Belliure and Clobert, 2004). CORT doses were calculated based

on the average growth of this species in the laboratory to avoid stress associated with measuring size each week (Freidenfelds et al., 2012) and ranged from 0.6 to 1 μg CORT/g body mass. A dosage in this range (0.8 μg/g body mass) resulted in circulating plasma CORT concentrations of approximately 77 ng/ml 30 min after application (Trompeter and Langkilde, 2011), which approximates plasma CORT concentrations in lizards 30 min following exposure to fire ants (61.90 ± 7.98 ng/ml; Graham et al., in press).

Lizards in the control treatment (n = 93) were placed in a sand-lined testing arena for 30 s and then had sesame oil applied to their back. This sham experimental group served as a control for handling and stress effects associated with captive housing and the experimental manipulations. We selected this complex control condition rather than a more simple non-handled control condition that would only have controlled for effects of captivity.

Eight to 18 weeks after the completion of treatments, once lizards had reached reproductive maturity (based on SVL; Parker, 1994; Tinkle and Ballinger, 1972), we conducted several assays of the physiological stress response. A unique subset of animals was used for each of the adult assays (described in subsequent sections). We assigned lizards to assays to balance for age, site of mother's origin, and sex (with priority given to age and site of mother's origin; Supplemental Table 1), and include these parameters in analyses (described in the Data analysis section).

2.4. Blood collection and stress response assays

We measured baseline and stress reactive concentrations of CORT following restraint for a subset of lizards (n = 32, Supplemental Table 1) on a single day that fell 8–14 weeks after treatments ended (52 to 57 weeks of age; variation due to differences in lizard hatching date). Baseline blood samples were obtained from the post-orbital sinus using 70 μl heparinized microhematocrit tubes (VWR, San Francisco CA) within 3 min of capture from their home enclosures (Romero and Reed, 2005). We then individually placed lizards in cloth bags for 30 min, after which we collected a second blood sample to assess CORT reactivity to this standardized restraint stressor (Romero and Reed, 2005).

We measured the CORT response to an ecologically-relevant stressor, attack by fire ants, for a separate subset of lizards (n = 31) on a single day 8–16 weeks after treatments ended (51 to 59 weeks of age). Lizards were placed inside a sand-lined arena containing 15–20 fire ants for 60 s after the first ant attacked. The number of ants that attacked lizards during these trials was similar across early life treatments (ANOVA: $F_{1,30}$ = 1.068, p = 0.358) and invasion status of the source population ($F_{1,30}$ = 0.041, p = 0.841; overall mean = 3.0 ± 1.2 SD ants). A separate subset of lizards (n = 32) was placed in a sand-lined arena with no ants for 70 s to serve as a control. The difference in duration of these fire ant versus control trials (60 vs. 70 s) reflects the average time it took for fire ants to attack after placing a lizard in the arena (Robbins, unpubl. data). At the completion of the trial, lizards in both the fire ant-exposure and control groups were immediately returned to their home enclosures for 60 min, after which we obtained a blood sample as described above. All blood samples were maintained on ice during collection, then centrifuged, and plasma drawn off and immediately frozen (−20 °C) until assays were performed.

2.5. ACTH challenge

To determine the ability of lizards' adrenal glands to mount a CORT response, we conducted an ACTH challenge on a separate subset of lizards (n = 67) over two days that fell 11 to 18 weeks after treatments ended (54 to 61 weeks of age). Lizards were injected intraperitoneally with 70 or 100 μl of either adrenocorticotrophic hormone (ACTH), a pituitary hormone that stimulates the adrenal glands to excrete CORT in lizards (n = 34), or saline solution as a control (n = 33). ACTH

(Sigma A6303, Saint Louis, MO) was dissolved in saline prior to injection (doses ranged from 0.56 to 0.80 IU (Klukowski, 2011), but ACTH doses did not significantly explain variation in CORT concentrations; see the Data analysis section). After injection, all lizards were placed in individual cloth bags for 60 min, after which we obtained a blood sample. Blood samples were processed using the previously described methods.

2.6. Hormone analysis

We measured plasma CORT concentrations using Corticosterone High Sensitivity EIA Kits (Immunodiagnostic Systems Ltd., Fountain Hills, AZ, USA) following directions provided in the kit. These kits have been validated for eastern fence lizards (Trompeter and Langkilde, 2011). We diluted plasma 1:9 with buffer (5 μl plasma: 45 μl buffer) to ensure that samples fell within the range of detection of the assay's standard curve. We ran all samples in duplicate. The mean intra-assay coefficient of variation within the six kits was 2.35% (1.53% to 2.91%), and the mean interassay coefficient of variation was 5.11%.

2.7. Data analysis

CORT concentrations at baseline, following ACTH- and saline-injection, and following exposure to fire ants or the associated control were log transformed prior to analysis to meet assumptions of parametric tests. One data point was omitted from analysis of baseline CORT and one from the analysis of CORT reactivity to restraint, as their values were >2 standard deviations from the mean.

For restraint stress, we calculated *CORT reactivity* as CORT concentration 30-min after stressor minus baseline CORT concentration. This provides an estimate of an individual's ability to increase CORT production on a short time scale in response to acute challenge, controlling for pre-stressor CORT levels. Because baseline CORT concentrations were not taken for lizards before exposure to fire ants or control arena or before ACTH- or saline-injection, thus we analyzed *CORT response* to these stressors as post-stressor concentrations. We analyzed 5 parameters of glucocorticoid production in adult lizards: 1) baseline CORT, 2) CORT reactivity to restraint, 3) CORT response to fire ant exposure and fire ant handling control, 4) CORT response to ACTH-injection, and 5) CORT response to saline-injection in five separate analyses using mixed-model ANCOVA (Table 1). We included early life treatment, fire ant invasion status, source population (nested within invasion status), and sex as factors, maternal ID as a random effect, and SVL, age, and bleed order as covariates. Time to bleed was included as a covariate in the model for baseline CORT, baseline CORT was included as a covariate in the model for CORT reactivity to restraint, and fire ant exposure assay (FA vs. handling control) was included as a factor in the model of CORT response to fire ant versus handling control exposure.

Sex, site, and age did not significantly explain variation in any of the CORT concentration data (p > 0.100); time to bleed, bleed order, and SVL did not significantly explain variation in baseline CORT concentrations (p > 0.098); SVL did not significantly explain variation in CORT reactivity to restraint or CORT responses to injection (p > 0.120); and the volume of ACTH or saline injected did not significantly explain variation in the CORT response (p > 0.831). These variables were thus omitted from the respective final models (Table 1). In cases where interactions were non-significant, they were removed from the final model to preserve statistical power (Table 1). For significant effects, Cohen's d (effect size) was estimated as the difference between least squares means divided by their pooled standard deviation (Thalheimer and Cook, 2002). All statistical analyses were performed using JMP (version 12.0, SAS Institute Inc., Cary NC) with α = 0.05.

Table 1
Parameters included in the full models for analyses of 5 parameters of glucocorticoid production in adulthood: baseline CORT, CORT reactivity to restraint, CORT response to fire ants or a handling control, CORT response to ACTH injection, and CORT response to saline injection. Parameters not included in the original models are greyed out, and those retained in the final models are indicated with an X.

	CORT Concentrations of Adult Lizards				
	Baseline	Restraint	Fire Ants or Control	ACTH	Saline
Maternal ID (*random*)	X	X	X	X	X
Treatment	X	X	X	X	X
Invasion status	X	X	X	X	X
Treatment × invasion					
Site (invasion)					
SVL			X		
Age					
Sex					
Bleed time					
Bleed order					
Baseline CORT		X			
Assay (FA vs. control exposure)			X		
Treatment × assay					
Assay × invasion status					
Treatment × assay × invasion status					
Volume injected					

3. Results

3.1. Baseline corticosterone

Neither early life treatment nor invasion status of the source population affected baseline CORT concentrations of adult lizards (Fig. 1; early life treatment $F_{2,20} = 1.54$, $p = 0.240$; invasion status $F_{1,6} = 2.09$, $p = 0.196$; early life treatment × invasion status $F_{2,14} = 0.71$, $p = 0.397$). A post-hoc power analysis indicates that 27 additional lizards (a doubling of sample size) would have been required to detect a significant effect of invasion status (Invaded > Uninvaded; power = 0.29, LSN = 59).

3.2. Corticosterone reactivity to restraint

The change in CORT following restraint (CORT reactivity) was not significantly affected by early life treatment (Fig. 1; $F_{2,10} = 0.29$, $p = 0.752$). However, CORT reactivity was significantly higher in offspring of lizards from fire ant-invaded populations than uninvaded populations ($F_{1,9} = 5.24$, $p = 0.048$, $d = 0.87$; baseline covariate $F_{1,15} =$

2.45, $p = 0.140$). The effect of population invasion status did not differentially influence effects of early life treatment on CORT reactivity (early life treatment × invasion status $F_{2,9} = 0.34$, $p = 0.718$).

3.3. Corticosterone response to fire ants

CORT concentrations were significantly higher in lizards following adult fire ant exposure compared to those placed in an empty arena (Fig. 2; fire ant exposure assay $F_{1,35} = 12.05$, $p = 0.001$, $d = 0.90$; SVL covariate $F_{1,44} = 10.50$, $p = 0.002$). There was an effect of invasion status of the maternal population, but not early life treatment, on overall CORT concentrations across the two adult fire ant assays, with CORT concentrations being higher in lizards from invaded sites (invasion status $F_{1,23} = 4.87$, $p = 0.038$, $d = 0.57$; early life treatment $F_{2,53} = 0.86$, $p = 0.430$). CORT responses to fire ant exposure were similar across maternal populations and early life treatments (invasion status × fire ant exposure assay $F_{1,25} = 0.08$, $p = 0.782$; early life treatment × fire ant exposure assay $F_{2,34} = 1.83$, $p = 0.176$; early life treatment × invasion status $F_{2,34} = 2.02$, $p = 0.148$; early life treatment × invasion status × fire ant exposure assay $F_{2,34} = 0.55$, $p = 0.584$).

3.4. Corticosterone response to ACTH challenge

CORT concentrations following ACTH-injection were not affected by early life treatment (Fig. 3; $F_{2,22} = 2.39$, $p = 0.115$) but were significantly higher in offspring from fire ant-invaded source populations than in those from uninvaded populations ($F_{1,17} = 7.60$, $p = 0.013$, $d = 0.97$). Early life treatment and invasion status did not interact to affect CORT concentrations following ACTH injection (invasion status × early life treatment $F_{2,17} = 1.41$, $p = 0.272$).

Injection of saline had a similar effect on CORT concentrations of lizards regardless of their early life treatment (Fig. 3; $F_{2,13} = 0.25$, $p = 0.786$) or the invasion status of the source population ($F_{1,8} = 0.81$, $p = 0.394$; early life treatment × invasion status $F_{2,9} = 0.18$, $p = 0.837$), suggesting that differences in ACTH responsiveness were not due to differential responses to handling and injection.

4. Discussion

We investigated the effects of early life and transgenerational exposure to stressors, and the interaction of these exposure histories, on HPA activity in adulthood. We found no effect of early life stress (weekly exposure to fire ant attack or CORT) on adult baseline CORT concentrations, CORT reactivity to restraint, or the CORT response to fire ants or
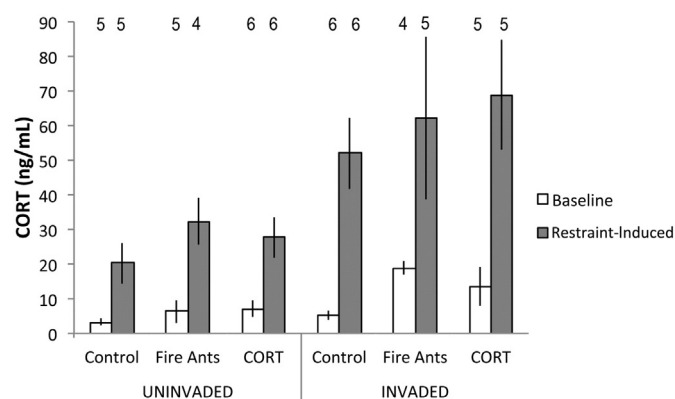

Fig. 1. CORT reactivity to restraint is higher in offspring of lizards from fire ant-invaded sites. CORT concentrations at baseline (white bars) and after restraint (shaded bars) of adult lizards that were exposed during early life to weekly fire ant attacks, exogenous CORT, or control treatment. CORT reactivity (post-restraint minus baseline) was assessed in the statistical model, but stress-induced concentrations are plotted here for ease of comparisons between graphs. Bars represent means ± one standard error. The sample size for each group is given above each bar.
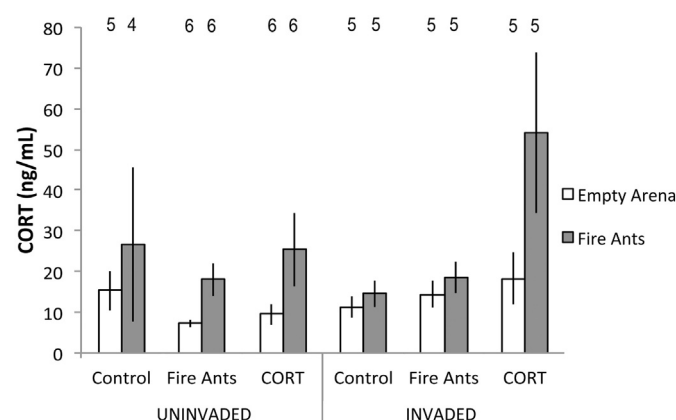

Fig. 2. CORT concentrations following adult fire ant exposure are similar in offspring of lizards from fire ant-invaded and uninvaded sites. CORT concentrations after adult exposure to an empty arena (white bars) or to fire ant attack (shaded bars) of lizards that were exposed during early life to weekly fire ant attacks, exogenous CORT, or control treatment. Bars represent means ± one standard error. The sample size for each group is given above each bar.

ACTH injection. Offspring of lizards from high-stress fire ant-invaded and low-stress uninvaded populations had similar CORT concentrations at lab baseline in adulthood, and similar CORT responses to fire ant exposure; thus these traits were not affected by different environments experienced across generations. However, transgenerational exposure to predatory stress did influence some measures of HPA reactivity: offspring of lizards from invaded populations had higher CORT responses to restraint and ACTH injection than did offspring of lizards from uninvaded populations. These results suggest that transgenerational history with stress has important effects on elevating adult HPA activity.

4.1. Baseline CORT

Neither early life stress nor transgenerational history with stress affected baseline CORT. Baseline CORT varies greatly within individuals as seasons and metabolic demands change (Bonier et al., 2009; Landys et al., 2006). Because this flexibility occurs on short time scales, variation in baseline CORT concentrations may be best explained by acute environmental conditions rather than genetic factors (Jenkins et al., 2014). The results of this current study suggest that field patterns of lizards with higher baseline CORT in fire ant-invaded vs. uninvaded sites (Graham et al., 2012) are likely not due to early life stress or transgenerational history with stress, but rather may be the result of an acute CORT response to recent and frequent fire ant attacks (Freidenfelds et al., 2012; Langkilde unpubl. data). This is supported by the fact that lizards from fire ant-invaded and uninvaded sites return to similar baseline CORT concentrations within one week in captivity (Langkilde, unpubl. data). Alternatively, these field differences may reflect a persistent increase in true baseline CORT that occurs as a result of CORT elevation due to frequent fire ant attack (Fig. 2; Graham et al., in press). Testing how long differences in baseline CORT between lizards from fire ant invaded and uninvaded sites persist in captivity would be informative in this regard. A power analysis of our data indicates that doubling our sample size would have allowed us to detect a significant effect of invasion status in baseline CORT production, with baseline CORT concentrations higher in offspring of lizards from invaded sites. This would match field patterns, and may suggest that baseline levels of CORT have a transgenerational component, though the mechanism is not clear.

4.2. Early life stress

We did not observe an effect of early life fire ant or CORT exposure on adult HPA activity. Early life stress does not affect adult baseline

Fig. 3. ACTH-induced CORT concentrations are higher in offspring of lizards from fire ant-invaded sites. CORT concentrations following injection with saline solution (white bars) or ACTH (shaded bars) of adult lizards that were exposed during early life to weekly fire ant attacks, exogenous CORT, or control treatment. Bars represent means ± one standard error. The sample size for each group is given above each bar.

concentrations of CORT in rats (Mirescu et al., 2004; Plotsky and Meaney, 1993), but does affect adult CORT reactivity in rats and humans (Carpenter et al., 2007; Gunnar et al., 2009; Liu et al., 1997; Matthews, 2002) and birds (Spencer et al., 2009). We also found no evidence of an effect of early life stress exposure on adult adrenal function, as evidenced by no treatment difference in CORT concentrations following ACTH injection. It is important to note that we measured HPA activity several months after early life treatments had ended. It is possible that early life stress exposure affected HPA activity during or immediately following the treatment period, but that any effects did not persist two months beyond the completion of treatments. This is counter to the literature documenting persistent effects of early life stress on adult behavior and physiology in rodents and humans (McCormick and Green, 2013; Meaney et al., 1994; Veenema, 2009). However, unlike rodents and humans, the lizard species studied here does not engage in maternal care, which may serve to exacerbate the effects of early life stress. Our lizards may have habituated to regular exposure to fire ants in the laboratory environment (Cyr and Romero, 2009; Romero, 2004; Romero et al., 2009). Alternatively, the fire ant colony may have become less venomous in captivity (Tschinkel, 2006; Xian-Fu et al., 2015), rendering the fire ant exposure treatment similar to the control. In the current study, lizards exposed to fire ants in adulthood had higher CORT concentrations compared to those exposed to an empty sand-lined enclosure, suggesting that attack by our fire ants still induced a CORT response. However, CORT responses to the fire ants used in the current study were lower than for lizards exposed to free-living fire ants (Graham et al., in press). We similarly found no effect of early life CORT treatment on HPA activity (however, increasing sample sizes may have provided power to detect an effect of the early life CORT treatment; Fig. 2). Future research on whether more frequent, intense, or varied duration of early life stress treatments produce lasting effects into adulthood would be informative (Busch et al., 2008; McCormick et al., 1998; McCormick et al., 2015; McEwen et al., 1997).

4.3. Transgenerational exposure to stress

Transgenerational exposure to stress predicted some aspects of adult HPA activity in the current study. Offspring of lizards from high-stress fire ant-invaded populations had higher CORT reactivity to restraint and ACTH injection than did offspring from low-stress uninvaded populations, irrespective of early life stress treatment. These results mirror the differences in adult HPA activity of these populations in the wild (Graham et al., 2012). Because these patterns were observed even in lizards from the control treatment, the current study indicates that field patterns of elevated CORT responsiveness in fire ant-invaded populations (Graham et al., 2012) are not strongly driven by within-lifetime stress, but rather by transgenerational mechanisms. This could take two forms:

a) At high-stress fire ant-invaded sites, natural selection may favor heightened CORT responsiveness of lizards, as elevated concentrations of CORT trigger important survival behaviors that allow these animals to escape attack from fire ants (Trompeter and Langkilde, 2011; Langkilde, unpubl. data). It is possible that transgenerational history with stress may select for greater sensitivity to early life stressors in these populations (Gariépy et al., 2002; Jenkins et al., 2014), although we did not see evidence of this in this study. Further research on how transgenerational and early life stress histories may interact in natural populations will increase our understanding of the ecological and evolutionary significance of these patterns.

b) Despite the fact that fire ant-invaded and uninvaded populations have experienced different stressors for >31 generations, maternal exposure to stressors, and not natural selection, may be driving these effects. Mothers from high-stress invaded populations may differentially provision their eggs (e.g. with nutrients, CORT; Hayward and Wingfield, 2004; Seckl and Meaney, 2004) or alter
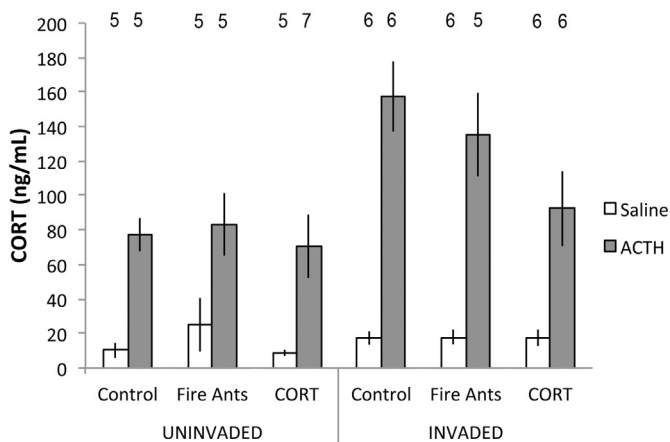
their behavior (e.g. feeding, thermoregulation, maternal care; Champagne and Meaney, 2001; Shine and Harlow, 1993). These changes may affect the perinatal environment and lead to epigenetic changes (Fish et al., 2006; Weaver et al., 2004), which can affect the stress responsiveness of offspring (maternal effects; Liu et al., 1997; Love et al., 2013, 2008) and prepare offspring for a stressful environment (maternal matching: Sheriff and Love, 2013). Mothers in this study were all captured while gravid, which may have had a homogenizing effect across treatments, and they were housed under relatively innocuous conditions in the lab, which may have reduced the potential to observe differences in offspring based on maternal effects. However, mothers had experienced different levels of stress due to fire ants in the field, including during pregnancy, and this field exposure should be adequate to induce maternal effects (Love and Williams, 2008), except those that occur close to laying. Prior research in this species argues against a role for increased yolk CORT in explaining elevated CORT reactivity; three month-old hatchlings from eggs treated with CORT had lower, not higher, CORT reactivity to restraint than those from eggs treated with oil vehicle only (McCormick, unpubl. data). Future research should investigate how these potential maternal mechanisms may affect adult HPA activity.

The higher CORT reactivity in offspring of lizards from high-stress fire ant-invaded populations is mirrored in our ACTH results: offspring of lizards from these invaded sites had higher CORT responses to ACTH compared to those from uninvaded sites. This indicates a general up-regulation of CORT reactivity in offspring from fire ant-invaded populations, with differences occurring at the adrenal glands rather than at the level of the hypothalamus or the pituitary (Seaward, 2006; Stratakis and Chrousos, 1995).

It is interesting to note that invasion history of the maternal population affected the CORT response of offspring to an ecologically-novel stressor (restraint) but not to an ecologically-relevant stressor (fire ant attack). Animals may become less sensitive to the specific stressor to which they are persistently exposed over time (acclimation), but more sensitive to novel stressors (facilitation; Romero, 2004). These changes typically occur within a lifetime (Romero, 2004), but it is possible that these could be transferred to subsequent generations (i.e. sensitized response to fire ants and heightened response to novel stressors transferred to next generation). In the current study, however, there was no evidence of within lifetime effects (no early life treatment effects). These results caution that researchers should consider the ecological relevance of experimentally applied stressors when performing these kinds of studies, as animals may respond in different ways to different types of stressors (Ariza Traslaviña et al., 2014; Koolhaas et al., 2011; Segerstrom and Miller, 2004). Further work is required to determine if and how offspring HPA reactivity is programmed by parental stressor exposure, and if this programming is specific to the type of stressors to which parents are exposed.

5. Conclusions

This study demonstrates that early life stressors alone may not drive population-level changes in adult physiology, as these may also be affected by transgenerational exposure to stress. In this system, we observed upregulation of CORT reactivity to some stressors in lizard offspring from sites invaded by predatory ants, which may have fitness benefits in these environments by triggering adaptive behavior. The exposure of an individual's ancestors to stress may thus be critically important in regulating physiological stress responses, particularly in populations that have been exposed to the same stressors over multiple generations. This has important consequences for predicting and managing the effects of stress and for establishing whether these effects can be reversed within an individual's lifetime.

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