Attracting unwanted attention: generalization of behavioural adaptation to an invasive predator carries costs

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ABSTRACT

Behavioural responses of animals to environmental cues are often governed by general “rules of thumb”. Animals that face novel conditions as a result of global environmental change may alter these behavioural rules to persist. However, adaptation of generalized rules to novel pressures may cause a species to be maladapted to original conditions (e.g., predators) that remain in its environment. Invasive red imported fire ants (*Solenopsis invicta*) are novel predators of eastern fence lizards (*Sceloporus undulatus*). Lizards from fire ant-invaded sites break crypsis to flee from fire ants at higher frequencies than fire ant naïve lizards. This shift promotes survival of attacks by these invasive ants but could result in attacks by native visual predators. Generalization of this increase in antipredator behaviour to native species could further increase this cost. We tested whether lizards’ increased propensity to flee from fire ants is generalized to native ants and a predatory bird. We found that increased behavioural responsiveness to fire ants was generalized to two native ants but not to a perceived avian predator. We also found that lizards from populations invaded by fire ants had higher prevalence of injuries in the field, likely indicating greater attempted predation. We propose that generalized anti-ant behaviour may improve survival in the presence of fire ants but increase attacks by native visual predators. This study suggests that generalized rules can be maladaptive under novel conditions and highlights the challenges of assessing the costs and benefits of adaptations to rapid environmental change.

Key-words antipredator response, invasive species, maladaptation, predator recognition, trade-off, crypsis, behavioural rule

23 The complex suites of behaviours used by many organisms are often a product of both
24 specific and general underlying rules. For example, individuals can use behavioural rules to
25 decide to treat as a potential mate or predator anything that smells, tastes, appears, or behaves
26 in a specific way (Jennions & Petrie, 1997; Frid & Dill, 2002). General rules may allow responses
27 to generic cues such as looming shapes (Carlile, Peters, & Evans, 2006) or objects of a particular
28 color, texture (Gwynne & Rentz, 1983), or size (Arak, 1983; Marco, Kiesecker, Chivers, &
29 Blaustein, 1998). Precise rules may require a particular combination of multiple, specific cues to
30 elicit a behavioural response (Hankiston & Morris, 2003).

31 Behavioural rules are generally only as complex as required to be adaptive within an
32 environment (Schlaepfer, Runge, & Sherman, 2002), and general rules may evolve more quickly
33 and easily than complex ones (Orr, 2000). While relying on specific rules reduces the risk of
34 making errors, general rules for behaviour are valuable as they allow for responses to many
35 different environmental cues and obviate the costs of discriminating specific cues (Sih, 2013;
36 Carthey & Banks, 2014). These rules are strategies that work on aggregate over multiple
37 iterations in diverse or uncertain situations (Lima & Dill, 1990), and their use may lead to more
38 predictable outcomes in variable environments (Heiner, 1983).

39 However, reliance on general rules can incur costs as the chances of responding
40 inappropriately to cues are higher than if rules are more specific. For example, males with
41 indiscriminate mate choice could waste time and energy, and lose mating opportunities, by
42 attempting to mate with unsuitable partners or objects (e.g., anurans mating with dead or
43 heterospecific individuals, or shoes; Meshaka, 1996), and parents caring for any offspring they

44 encounter could bear the costs of raising unrelated individuals (e.g., hosts of nest parasites;
45 Payne, 1998).

46 The fitness consequences of generalized behavioural responses are a focus of much
47 recent research and theory, especially in the context of rapid environmental change
48 (Schlaepfer, Sherman, Blossey, & Runge, 2005; Sih, 2013, Carthey & Banks, 2014). Novel or
49 rapidly changing selective regimes can result in maladaptation or evolutionary traps (Crespi,
50 2000; Schlaepfer, Sherman, & Runge, 2010), including in response to environmental change
51 (Bradley St. Clair & Howe, 2007, Sinervo et al., 2010; Kerby & Post, 2013). Indeed, general rules
52 of behaviour can be especially costly when the environment changes and organisms apply these
53 rules in the wrong context. For example, an organism could suffer reduced fitness by
54 attempting to mate with an invasive species (Gröning & Hochkirch, 2008) or man-made object
55 that fulfils their general rules for mate identification (Gwynne & Rentz, 1983). Generalized
56 responses to novel predators and prey can also reduce survival. For instance, generalist
57 predators preying upon toxic invasive Cane Toads (*Rhinella marina*) can be poisoned (Shine,
58 2010), and New Zealand mudsnails react to invasive crayfish by burrowing, an ineffective
59 defence against these novel predators (Sih, 2013). Accelerating global environmental change
60 provides an excellent opportunity to examine the costs and benefits of generalized behaviours,
61 especially in the case of adaptive responses to the novel conditions (Sinervo et al., 2010;
62 Carthey & Banks, 2014).

63 To fully determine the consequences of behavioural adaptations to novel selective
64 pressures, we need to understand:

- 1) *Behavioural change*. How behaviour has changed in response to novel environmental conditions.
- 2) *Specificity of change*. If behavioural changes are specific to novel environmental conditions (e.g., to an introduced predator only), or generalized to similar stimuli (e.g., to other ecologically similar species) or even more broadly (e.g., to all potential predators).
- 3) *Costs of change*. If behavioural changes are either beneficial or costly (e.g., cause decreased survival or wasted time and energy), as we might expect if changes in behaviour are generalized across multiple situations/species.

Antipredator behaviour is an excellent system with which to address these issues. These behaviours are expected to be under strong selection in many situations including the introduction of novel predators (Abrams, 2000). For example, native tadpoles (*Lithobates aurora*) that are naïve to invasive predatory bullfrogs (*Lithobates catesbeiana*) suffer high mortality (Kiesecker & Blaustein, 1997). However, tadpoles from populations that have been exposed to this novel selection have adapted in less than 60 years by reducing activity levels to avoid predation (Kiesecker & Blaustein, 1997).

Even when successful, antipredator behaviour is known to carry a variety of costs, including higher energy usage, lower foraging rates/opportunities, and reduced ability to defend territories (Lima & Dill, 1990; Martín, Luque-Larena, & Lopez, 2009). If adaptations to novel predators alter general antipredator behavioural rules, applying these rules to interactions with native predators may incur these costs and lower fitness. When a trade-off between the costs and benefits of novel behaviour is not optimally balanced, maladaptation,

potentially as an ecological or evolutionary trap, may occur (Schlaepfer et al., 2002; Schlaepfer et al., 2005; Sih 2013).

Here, we use a system involving change in antipredator behaviour of native lizards (*Sceloporus undulatus*) in response to invasive, predatory red imported fire ants (*Solenopsis invicta*; hereafter fire ants) to address the three issues highlighted above:

1) *Behavioural change*. Antipredator behaviour of this lizard has changed in response to invasion by fire ants: fence lizard populations largely switch from relying on crypsis (an adaptive response to predominantly visual predators; Martín et al., 2009) to flight during encounters with these predatory invaders (Langkilde, 2009a).

2) *Specificity of change*. In this study, we use staged encounters to test if this shift in behavioural response to fire ants is generalized to two native ant species that are prey for fence lizards, and to a native avian predator, revealing the specificity of this behavioural adaptation.

3) *Costs of change*. We also look for evidence of greater prevalence of injuries in lizards from populations showing increased responsiveness to fire ants, a potential fitness cost of this behavioural change.

METHODS

Study System

Fire ants are native to South America and were introduced to Mobile, AL in the southeastern United States in the 1930's (Tschinkel, 2006). This species has since spread throughout the southeastern U.S. where it overlaps with the range of the eastern fence lizard (Conant & Collins, 1998). While fence lizards and fire ants both favor disturbed, edge habitats

(Langkilde, 2009b) and interact frequently in natural environments (Freidenfelds, Robbins, & Langkilde, 2012), fence lizards do not change their habitat use in the presence of fire ants (Langkilde, 2009b). Fire ants recruit in high numbers to potential prey, including small vertebrates (Allen, Demarais, & Lutz., 1994; Wojcik et al., 2001), and as few as 12 attacking ants can paralyze and kill an adult fence lizard (Langkilde, 2009a). Ants make up a significant portion of the diet of fence lizards (DeMarco, Drenner, & Ferguson, 1985; Mobley, 1998), and lizards can be envenomed when consuming fire ants (Langkilde & Freidenfelds, 2010), which become the numerically dominant ant within invaded areas (Vinson, 1994; Epperson & Allen, 2010).

Fence lizards have adapted morphologically and behaviourally to invasive fire ants within 70 years (≈ 40 generations; Langkilde, 2009a). These lizards are well-camouflaged and generally rely on crypsis to defend against visual predators including lizards, snakes, and birds of prey (Cooper, Burghardt, & Brown, 2000; Jensen, Camp, Gibbons, & Elliott, 2008). While lizards from populations not yet invaded by fire ants (uninvaded sites) flee from $\approx 50\%$ of fire ant attacks, lizards from populations invaded by fire ants for long periods of time (invaded sites) flee at a higher rate ($\approx 85\%$; Langkilde, 2009a). These flee behaviours reduce contact with and envenomation by attacking fire ants (Freidenfelds et al., 2012).

Study Sites and Animals

We captured lizards from six study sites in the southeastern United States: (1) Solon Dixon Forestry Education Center, Escambia Co., Alabama (31.16° , -86.70°), (2) Geneva State Forest, Geneva Co., Alabama (31.12° , -86.17°), (3) Historic Blakeley State Park, Baldwin Co., Alabama (30.74° , -87.91°), (4) Saint Francis National Forest, Lee Co., Arkansas (34.71° , -90.73°), (5) Edgar Evins State Park, Dekalb Co., Tennessee (36.08° , -85.83°), and (6) Standing Stone State

Park, Overton Co., Tennessee (36.47°, -85.42°) (all coordinates in WGS84). Sites 1, 2, and 3 were first invaded by fire ants approximately 74, 55, and 82 years ago, respectively, while sites 4, 5, and 6 have not been invaded by fire ants (Callcott & Collins, 1996). All sites are characterized by temperate mixed forests and abundant edge and disturbed habitats where both fence lizards and fire ants are found at their highest densities (Langkilde, 2009b; Langkilde, unpublished data). Only adult fence lizards were collected for behavioural trials, as juvenile lizards flee from fire ant attack at high rates regardless of population of origin (Langkilde, 2009a). Following capture, lizards were measured for mass (to the nearest g) and snout-vent length (SVL, to the nearest mm). They were housed in mixed sex groups (3-4 lizards) in plastic enclosures (56 X 40 X 30 cm, L X W X H) furnished with a water dish and shelter. A 60-W incandescent lamp at one end of the enclosure created a thermal gradient allowing lizards to thermoregulate. Lizards were provided water *ad libitum* and fed crickets (*Acheta domesticus*) dusted with supplementary minerals and vitamins four times weekly (Herptivite and Ultrafine Calcium with Vitamin D; Repcal, Los Gatos, CA).

We used these lizards to test whether the difference in behavioural response to fire ants following invasion is generalized to native species - ants that provide similar stimuli to fire ants but do not pose a lethal threat (Buschinger & Maschwitz, 1984; Wilson, 2003; Thawley, personal observation), and a native avian predator to which lizards should generally respond by maintaining crypsis (Parker, 1994).

Behavioural Response to Ants

We tested lizards from invaded (n=55) and uninvaded sites (n=43) for their fleeing response to fire ants and two species of native ants (*Dorymyrmex sp.* and *Pheidole sp.*) that

serve as food for fence lizards between 2011 and 2014. While these native ants will recruit to lizards, they have lower densities, recruit less aggressively, and have less potent venom than fire ants (or lack venom altogether), resulting in lower danger to fence lizards (Wilson, 2003; Tschinkel, 2006). Behavioural tests followed a protocol described in Langkilde (2009a). Briefly, we tethered lizards to pegs with 1 m lengths of cotton thread tied loosely around their necks. Pegs were placed in the ground 30 cm from a target ant mound, allowing the lizard to flee from ant attack but preventing escape into the surrounding landscape. Temperature of mounds and lizards was determined using a Raytek MT-6 infrared thermometer (Raytek Corporation, Santa Cruz, California, USA) accurate to $\pm 0.2^{\circ}\text{C}$. Target mounds were gently disturbed to bring ants to the surface, and lizards were placed onto undisturbed portions of focal mounds such that disturbed ants could find lizards. Trials ended after 60 seconds or when a lizard fled beyond the boundary of the ant mound. We recorded the time at which the lizard fled from the mound and the average number of ants on the lizard. All lizards were tested against all three ant species on consecutive days, with the order of ant species being randomized.

We analyzed the flight response of lizards to the three species of ant using a generalized linear mixed model with a logit link. Our initial model included invasion status (invaded or uninvaded), sex, treatment (ant species), trial order, year, and interactions (invasion status*treatment, invasion status*sex, and invasion status*order) as fixed effects; site nested within invasion status as a random effect; snout-vent-length (SVL), mass, lizard temperature, mound temperature, and number of attacking ants as covariates; and a random effect for individual identity to account for repeated measures from individual lizards. Our final model included invasion status, sex, ant species, and trial order as fixed effects; site nested within

invasion status as a random effect; and SVL and lizard temperature as covariates. Year, interactions between fixed effects, mass, mound temperature, and number of attacking ants did not significantly explain variation in the behavioural response of lizards and were omitted from the final model. Satterthwaite's Approximation was used to determine denominator degrees of freedom for F-tests (Keselman, Algina, Kowalchuk, & Wolfinger, 1999).

Behavioural Response to an Avian Predator:

We filmed behavioural responses of lizards from three fire ant invaded sites (n=59) and three sites uninvaded by fire ants (n=41) to a simulated avian attack in an experimental arena in 2013. The arena (56 X 40 X 30 cm, L X W X H) was furnished with a sand substrate and a hollow, half-log shelter in the center of the arena which lizards could perch on and seek refuge beneath. We used a preserved adult American Kestrel on loan from the Penn State Ornithological Collection as a predator model (Federal Salvage Permit MB028785-0; PA Permit SAL00436). American Kestrels (*Falco sparverius*) are common, native predators of sceloporine lizards (Knowlton & Stanford, 1942; Craig & Trost, 1979), and have been used in antipredator behavioural studies with sceloporine lizards (Fine, 1999). The preserved kestrel was positioned in an attack posture with wings slightly spread, and we attached realistic cardboard eyes to better simulate a living predator, as lizards use the appearance of eyes to gauge the danger presented by a predator (Hennig, 1977). The kestrel bore an inconspicuous wire harness attached to a fixture on the ceiling directly above the test arena with clear monofilament line. This allowed a clearance of 10 cm between the kestrel and the top of the lizard's perch site in the experimental arena. Pilot data revealed that this setup produced a behavioural response in

≈50% of focal lizards. A blind hid observers throughout the trials and hid the kestrel from the focal lizard prior to a simulated attack.

Each day we randomly selected an untested lizard from each housing enclosure for testing to prevent stress associated with prior lizard handling in the same enclosure from influencing results. Each lizard was tested once. All trials were conducted within one minute of first handling the focal lizard to ensure that elevated stress due to handling did not affect responses (Romero & Reed, 2005). To commence a behavioural trial, we measured the focal lizard's temperature as described previously and placed it in the experimental arena. We standardized each focal lizard's position by placing it lengthwise on the perch, facing the direction from which the predator would appear and ensuring that all four feet were in contact with the perch. We then retreated behind the blind and released the kestrel. The kestrel passed over the perch four times (twice from each direction).

We recorded trials using a small webcam positioned within the blind and connected to a laptop computer. A small light, out of the lizard's visual range, indicated the release of the kestrel and was used to assess time to respond. We used Media Player Classic v1.5.2 (mpc-hc.org) to quantify three behavioural variables for each trial: (1) whether or not a lizard behaviourally responded to the simulated attack, (2) latency to response (time between kestrel release and initiation of a response by the focal lizard), and (3) the strength of the response. Response strength was assigned one of four levels: 0 - no reaction; 1 - weak reaction (the lizard ducked its head or flattened its body to the perch, but did not move more than two of its feet); 2 - strong reaction (the lizard moved three or more of its feet and substantially shifted its

position, usually onto the side of the half-log, but remained on the perch); and 3 - flight (the lizard completely left the perch, either seeking shelter beneath it or moving onto the substrate).

We tested for differences in probability of a lizard responding to simulated avian attack using logistic regression with invasion status (invaded or uninvaded) and sex as fixed effects and SVL and lizard temperature as covariates. For the subset of lizards that did respond to the simulated avian predator (n=53), differences in latency to respond were assessed using a general linear model with invasion status and sex as fixed effects and SVL and lizard temperature as covariates. We tested for differences in strength of response using ordinal logistic regression with invasion status and sex as fixed effects and SVL and lizard temperature as covariates. As latency to respond and strength of response were unrelated (Spearman's rho: -0.126, $p > 0.373$), we analyzed these two responses separately.

Injury Prevalence

We recorded evidence of injury, including missing limbs or parts of limbs, broken or regenerated tails, and scars for lizards, from three fire ant invaded and three fire ant uninvaded sites in 2011-2014 (n=589). These are the same sites as those from which we obtained the lizards used for behavioural trials. Injuries, including broken or regenerated tails, have been used for lizards as proxies for predation pressure (Arnold, 1988; Parker, 1994; but see Angilletta et al., 2004). We did not consider missing digits as injuries, as digits are lost outside of predatory interactions during intraspecific competition, aggressive courtship, or shedding (Schoener & Schoener, 1980).

We tested for differences in prevalence of injury using a generalized linear mixed model with a logit link with an initial model including invasion status (invaded or uninvaded), sex,

interactions (invasion status*sex and invasion status*SVL), and year as fixed effects, site nested within invasion status as a random effect, and SVL and mass as covariates. Interactions between fixed effects (above) and lizard mass did not significantly explain variation in the data as determined by likelihood ratio tests and were omitted from the final model. This model for injury prevalence thus included invasion status and sex as fixed effects, site nested within invasion status as a random effect, and SVL as a covariate. A model selection approach comparing AICc values of alternative *a priori* models also resulted in the selection of this same best fit model. All analyses were conducted using SPSS v.21 (IBM Corp., 2012).

Ethical Note

No lizards were killed during trials or exhibited sublethal effects of exposure to fire ants such as loss of muscle tone or lethargy. Lizards were returned to points of capture where permitted; where release was not permitted, lizards were euthanized and accessioned as specimens at Pennsylvania State University. Animal collection was permitted by the respective states: Alabama (Permit #'s: 2011000058268680, 2012000086668680, 2013000092668680, 2014070574868680); Arkansas (Permit #'s: SO-FW-FY10-05, SO-FW-FY12-09, 041120111, 040420122, 040420133, 051520141), and Tennessee (Permit #'s: 2010-025, 3574). Research protocols were approved by the animal ethics committees of Pennsylvania State University (Protocol # 44595 and 35780) and Auburn University (Protocol # 2011-1922).

RESULTS

Behavioural Response to Ants

Lizards from fire ant uninvaded sites fled less frequently from encounters with native ants and invasive fire ants than did lizards from fire ant invaded sites (Invasion Status:

$\beta_{\text{Uninvaded}} = -1.469$, $F_{1,4} = 13.799$, $P < 0.018$; Fig. 1A). The odds of lizards from invaded sites fleeing were 4.3 times higher than those from uninvaded sites. Lizards from both invaded and uninvaded sites showed differences in frequency of flee responses to different species of ants (Ant Species: $F_{2,285} = 9.111$, $P < 0.001$; Invasion Status*Ant Species: $F_{2,283} = 1.484$, $P > 0.229$; interaction not included in final model). Lizards fled less from the *Pheidole* sp. of ant than from the other two ant species (Ant Species: $\beta_{\text{Pheidole}} = -1.404$, $P < 0.001$; Sex: $F_{1,93} = 4.505$, $P < 0.036$; SVL: $F_{1,95} = 6.935$, $P < 0.01$; Lizard Temperature: $F_{1,285} = 7.522$, $P < 0.006$; Trial Order: $F_{2,285} = 3.924$, $P < 0.021$).

Behavioural Response to Avian Predators

Lizards from fire ant invaded and uninvaded sites were similarly likely to respond to the model avian predator (Fig. 1B), had a similar latency to respond (Fig. 2), and their response was of similar strength (Fig. 3) (Response to avian predator, Invasion Status: Wald $\chi^2_1 = 0.027$, $P = 0.577$; Sex: Wald $\chi^2_1 = 0.038$, $P = 0.845$; SVL: Wald $\chi^2_1 = 1.527$, $P = 0.216$; Lizard Temperature: Wald $\chi^2_1 = 0.227$, $P = 0.634$) (Latency to respond, Invasion Status: $F_{1,51} = 0.882$, $P = 0.352$; Sex: $F_{1,51} = 0.139$, $P = 0.711$; SVL: $F_{1,51} = 0.090$, $P = 0.765$; Lizard Temperature: $F_{1,51} = 0.001$, $P = 0.982$) (Strength of response, Invasion Status: Wald $\chi^2_1 = 0.027$, $P = 0.868$; Sex: Wald $\chi^2_1 = 0.017$, $P = 0.897$; SVL: Wald $\chi^2_1 = 0.188$, $P = 0.665$; Lizard Temperature: Wald $\chi^2_1 = 0.059$, $P = 0.809$).

Injury Prevalence

Lizards from fire ant uninvaded sites had lower injury prevalence than did lizards from invaded sites (Invasion Status: $\beta_{\text{Uninvaded}} = -0.778$, $F_{1,585} = 13.799$, $P < 0.001$; Sex: $\beta_{\text{q}} = -0.911$, $F_{1,585} = 19.718$, $P < 0.001$; SVL: $\beta_{\text{SVL}} = 0.607$, $F_{1,585} = 13.166$, $P < 0.001$; Fig. 4).

Fig. 1 Percent of eastern fence lizards (*Sceloporus undulatus*) from fire ant invaded (dark red) and uninvaded (light blue) populations that fled from a) attack by two genera of native ants and invasive fire ants (estimated marginal means calculated for SVL = 6.4 cm and lizard temperature = 30.7°C) and b) simulated attacks by an avian predator (estimated marginal means calculated for SVL = 6.5 cm and lizard temperature = 33°C). Bars represent means ± 1 standard error.

Fig. 2 Latency of eastern fence lizards from fire ant uninvaded (light blue) and invaded (dark red) sites to respond to simulated attack by a model Kestrel, an avian predator of this species. Estimated marginal means calculated for SVL = 6.6 cm and lizard temperature = 32.7°C. Bars represent means ± 1 standard error.

306

307 **Fig. 3** Proportion of fence lizards from fire ant uninvaded (light blue; n=21) and invaded (dark
308 red; n=32) populations responding to simulated avian attack with weak (1), moderate (2), and
309 strong (3) escape behaviours (see text for a description of behaviours).

Fig. 4 Proportion of female and male fence lizards from fire ant uninvaded (light blue) and invaded (dark red) populations exhibiting injuries. Estimated marginal means calculated for SVL = 6.4 cm. Bars represent means ± 1 standard error.

DISCUSSION

The results of this study reveal that the increased use of fleeing behaviour by fence lizards following the invasion of fire ants is not restricted to encounters with this invader, but rather is generalized to native ants. This altered responsiveness is not extended, however, to encounters with avian predators. Higher injury prevalence in lizards from fire ant invaded sites suggests that lizards may suffer increased predation attempts as a cost of this behavioural shift, though the exact cause of this difference is not known.

These results extend findings that fence lizards from fire ant invaded sites flee from fire ants at a higher rate than do lizards from uninvaded sites (Langkilde, 2009a; Freidenfelds et al., 2012) by showing that these invaded site lizards are also more likely to flee from native ants (Fig. 1A). Despite the lesser threat posed by native ants, lizards from populations exposed to fire ants show an increased rate of fleeing from all three ant species included in our trials, suggesting that this response is generalized at least to ants. This generalization could be due to the sensory cues lizards use to respond to ants. Visual diagnosis of ant species can be difficult even for humans (Ward, 2010), and fence lizards have been observed consuming fire ants on their mounds when ant activity is low (Long, 2013), despite potentially lethal consequences (Robbins & Langkilde, 2012). However, these lizards often initiate flight after ants crawl on their bodies even without being stung (Thawley, personal observation), suggesting that lizards may respond to tactile cues that are present when ants defend their mounds. Like visual cues, these tactile cues are likely similar across ant species.

If lizards cannot reliably distinguish fire ants from native ants before being stung, fleeing from all species of ant may be the only behavioural strategy currently available to these lizards

to ensure they avoid fire ant envenomation. However, fewer lizards fled from one of the native ant species (*Pheidole* sp.; Fig. 1A), suggesting that they may be able to distinguish between ant species under some conditions. It is possible that different ant species vary in the amount of cue they provide (e.g., more or less of the same tactile contact), allowing differential responses by lizards. Fence lizards from fire ant invaded sites, but not uninvaded sites, avoid the scent of fire ants (Langkilde, 2009b), suggesting that fence lizards possess at least one pathway allowing identification of fire ants. If fence lizards can use relevant cues to discriminate fire ants from native ants, future adaptation may allow differential behavioural responses to different species of ants (Sih, Ferrari, & Harris, 2011).

In contrast to the generalization of behaviours across ant species, we found no evidence that the differences in behavioural response to ants between lizards from invaded versus uninvaded sites are generalized to avian predators (Figs. 1B,2,3). Lizards have a long evolutionary history with native avian predators, which pose a threat to lizards regardless of fire ant presence. Birds are ecologically distinct from ants and provide different cues, which may facilitate differential behavioural responses. Further research on what cues (tactile, visual, chemosensory) lizards use to detect predators would shed light on how they may evaluate avian and ant predators (e.g., Cantwell & Forrest, 2013) or distinguish between dangerous and non-threatening animals, including ants (see Suarez, Jon, & Case, 2000 for an example of a lizard distinguishing between ant species).

The generalized behavioural response to ants we observed is likely beneficial on average. In fire ant invaded areas, fence lizards encounter fire ants frequently due to their high relative abundance (Morrison, 2002; Freidenfelds et al., 2012). In these interactions,

misidentifying a fire ant as a native ant could be lethal, and fleeing from all ants as if they were fire ants is likely an adaptive strategy (Freidenfelds et al., 2012). However, fleeing breaks crypsis, a common defense to decrease detection by visual predators (Lima & Dill, 1990). Lizards that behaviourally respond to ants may thus risk predation by visual predators; a survival cost that has been suggested for other species interacting with invasive fire ants (Allen, Demarais, & Lutz., 1997; Long, Conner, Smith, & McCleery, 2015).

Our field data suggest a predation cost to this anti-ant behaviour as lizards at invaded sites show higher prevalence of injuries. The fact that males exhibit higher injury prevalence than females supports the interpretation of injury prevalence as reflecting predation attempts. Males select perch sites that improve territorial defence and courtship opportunities (Pounds & Jackson, 1983; Thawley, personal observation) but likely expose them to greater predation risk (Parker, 1994). Injury can itself increase risk of mortality and, in the case of tail loss, result in greater vulnerability to predators (Wilson, 1992; Downes & Shine, 2001), increasing potential fitness costs. The observed pattern of greater injury prevalence in fire ant invaded sites may be due to increased responsiveness to ants, or to a change in the behavioural response of these lizards to stress. Lizards from fire ant invaded sites are less likely to seek refuge underneath cover objects when stressed in the lab, a strategy that may help them avoid attack by fire ants at a cost of increased exposure to native predators (Trompeter & Langkilde, 2011).

It is important to note that the observed differences in injury prevalence are observational and could be explained by factors other than lizard behavioural differences. Increased injury prevalence at fire ant invaded sites could reflect a greater diversity or absolute abundance of predators in southern regions (Pianka, 1970). While we lack data on abundances

of native predators at study sites, habitats at these sites are ecologically similar and should share similar predator communities (Langkilde 2009a). Additionally,, lizard populations farther north than those at the study sites do not show lower injury prevalence (Thawley, unpublished data), arguing against a latitudinal cline in predation pressure in this instance (e.g., Pianka, 1970). Alternatively, this pattern could result from higher survival of predatory attacks by lizards at fire ant invaded sites resulting in higher rates of injury. We consider this possibility unlikely, however, as lizards from all sites flee from avian predators at similar rates (Fig. 1B) and with similar strengths and latencies to react (Figs. 2 and 3).

Together, these data suggest that this generalized behavioural reaction to ants may be maladaptive in the presence of native ants. Maladaptation due to biological invasions may be common, as previously adaptive responses to native species can reduce fitness when employed against invaders (Schlaepfer et al. 2002; Schlaepfer et al., 2005; Sih 2013). In this case, fleeing from fire ants is an effective defence and should improve lizard survival and fitness, even in the presence of native predators (Freidenfelds et al., 2012). However, because lizards that are adapted to fire ants generalize flight responses to non-lethal native ants, they likely experience increased attacks by visual predators, increased energy expenditure, lost feeding opportunities by fleeing from potential prey (native ants), and reduced ability to defend territories (Lima & Dill, 1990; Parker, 1994; Mobley, 1998). Future adaptations may support more optimal behavioral responses, such as fleeing only from dangerous ants, as selection should favor the incorporation of new cues or other refinements of generalized behavioural rules that promote higher fitness (Schlaepfer et al., 2010).

Future research into changes in behaviour triggered by novel threats should not only document the existence of responses to these pressures, but also assess whether these adaptations are generalized to other contexts. Some research suggests that generalized adaptations may better allow for adaptive responses to novel conditions when compared to more specific adaptations, which may be inappropriate or ineffective in novel situations (Carthey & Banks, 2014, but see Sih, 2013). However, our research shows that generalized adaptations are also vulnerable to being maladaptive under some types of rapid environmental change. Indeed, as anthropogenic change accelerates, disentangling the multiple drivers of behavioural and other adaptations, as well as assessing their costs and benefits, is likely to become increasingly challenging (Caro & Sherman, 2011). Understanding whether novel adaptations are generalized to multiple contexts will be critical for determining the long-term consequences of adaptive responses to environmental change and for managing affected species.

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