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THE EFFECT OF RED IMPORTED FIRE ANT VENOM ON WHOLE BODY
PERFORMANCE AND ERYTHROCYTE LYSIS OF EASTERN FENCE LIZARDS,
SCELOPORUS UNDULATUS

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ABSTRACT

Invasive species are a worldwide threat, contributing to the disruption of ecosystems and reductions in native biodiversity. Native species that are faced with ecologically novel invasive species may be under strong selective pressure to adapt to the new threats these invaders posed. Some native species, however, may be pre-adapted to deal with invasive threats due to existing adaptations to native species. The Red Imported Fire Ant, *Solenopsis invicta*, was introduced into the southeastern U.S. over 75 years ago and has since spread northward. *Solenopsis invicta* are venomous predators that can quickly paralyze and kill even large vertebrates. Native Eastern Fence Lizards (*Sceloporus undulatus*) from *S. invicta*-invaded sites have altered morphology and behavior that allow them to survive encounters with these invasive ants. These lizards, however, do not appear to have developed increased tolerance to fire ant venom. We test whether this apparent lack of adaptation is due to pre-adaptation to the venom of two native fire ant species (*Solenopsis geminata* and *Solenopsis xyloni*). We examined the effects of fire ant venom on the whole-body performance (tested via righting ability) and blood lysis of *Sceloporus undulatus* from *S. invicta*-invaded and uninvaded populations in the southern U.S., where these lizards overlap with native fire ants, and compare these to effects of fire ant venom on lizards from three northern sites that are outside the native fire ants' ranges. We found no evidence that Eastern Fence Lizards have evolved increased physiological resistance to fire ant venom: the impact of fire ant venom on righting ability and blood lysis did not differ between *S. invicta*-naïve versus experienced populations, or between lizard populations with or without historical exposure to fire ants. Testing for effects of prior exposure to closely related and ecologically-similar native species can provide insight into the prevalence of adaptation and importance of pre-adaptation, of native species to invasive threats.

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Chapter 1

Introduction

The world is in a state of rapid change, manifest through the abundance and spread of invasive species, the dramatically changing climate, and the anthropogenic and natural destruction of habitats (Abrahamson, 1989; Travis, 2003; Whitfield Gibbons et al., 2000). These changes provide valuable opportunities to examine evolution in action by examining adaptations of species to varying environmental conditions (Travis, 2003). Invasive species can impose strong selective pressure on populations of organisms native to the environments to which these native taxa are introduced (Phillips & Shine, 2006; Strauss, Webb, & Salamin, 2006). The impacts of invasive species are generally strong because invaders typically have moved long distances and are ecologically dissimilar to other native species, and so native species are usually not adapted to these novel threats (Strauss, Webb, & Salamin, 2006). Such a trend can be seen in the intentional introduction of Small Indian Mongoose (*Herpestes javanicus (auropunctatus))* to the West Indies and of the Nile Perch (*Lates niloticus*) to Lake Victoria, and the accidental introduction of the Brown Tree Snake (*Boiga irregularis*) to Guam and the Cane Toad (*Bufo marinus*) to Australia (Lowe, Browne, Boudjelas, & De Poorter, 2004; Phillips & Shine, 2006), all of which have negatively impacted several native species. Native species unable to adapt to an invader may suffer declines in population size or go extinct, while those that adapt will be more likely to persist under these new conditions.

The threat or impact of invaders on native species could, however, be tempered by the presence of a similar or related native species. Historical exposure to species that are ecologically similar to an invader could pre-adapt native fauna to the new, invasive threat, reducing its impact.

For example, resistance to the toxins of the Cane Toad (*Bufo marinus*) in Australian Keelback snakes (*Tropidonophis mairii*) is likely not due to rapid evolution in response to the invasive toad, but rather to pre-adapted resistance to the toxin via the Keelback snake's historical coexistence with related Bufonids in Asia (Llewelyn et al., 2010). Therefore, in order to predict how native organisms will be impacted by the introduction of a new species, it is important to consider the effects of pre-adaptation. This can be tested by including populations of the native species of interest that are outside the range of both the invasive threat and ecologically-similar native species.

The Red Imported Fire Ant (*Solenopsis invicta*) was accidentally introduced to the United States via Port Mobile, Alabama in the early 1930s (Callcott & Collins, 1996). Since introduction, this species has spread across the southern United States, and now inhabits over 106 million hectares of land (Vinson, 1997). The Eastern Fence Lizard (*Sceloporus undulatus*) co-occurs with the red imported fire ant across much of the invasive range, but there are many populations of fence lizards that have not yet been exposed to this invasive threat, which can be used as baselines for testing the impact of this invader (Conant & Collins, 1998; Langkilde, 2009a). Where these species do co-occur, they share similar habitats and often encounter each other while foraging (Langkilde, 2009ab).

This invasive fire ant has powerful venom that is used for prey capture, mound defense and territorial bouts (Haight & Tschinkel, 2003). Red Imported Fire Ant venom is composed mainly of organic alkaloids (MacConnell, Blum, & Fales, 1971) that have a variety of functions ranging from inducing hemolysis (Boronow & Langkilde, 2010) to disrupting nervous system functioning, the latter of which can result in paralysis or death at high doses (Yeh, Narahashi, & Almon, 1975; Yi et al., 2003; Howell et al., 2005; Langkilde, 2009a). Two native fire ant species, *S. geminata* and *S. xyloni*, co-occurred with *S. undulatus* in the south prior to the introduction of the red imported fire ant (Vinson, 1997), and have venom that while quantitatively

different from that of the red imported fire ant, has many components in common (Brand, Blum, Fales, & MacConnell, 1972). *S. xyloni* has been shown to kill the eggs and young of several bird species across its range (Hooper-Bui, Rust, & Reierson, 2004) and *S. geminata* can subdue small vertebrates with its sting (Holway, Lach, Suarez, Tsutsui, & Case, 2002) and has been linked to decreasing the nesting success of northern bobwhites in the southern United States (Travis, 1938).

Ants make up a large portion of the Eastern Fence Lizard's diet (Parker, 1994). Those that eat the Red Imported Fire Ant may get stung inside the mouth, which can be lethal (Boronow & Langkilde, 2010; Langkilde 2009a). Although covered in scales, *S. undulatus* are also susceptible to attack by fire ants, which lift a lizard scale with their mandibles and envenomate a lizard by repeatedly stinging the soft underlying skin (Langkilde, 2009a). As few as 12 stinging ants are able to paralyze and kill an adult lizard within a minute (Langkilde 2009a), and eating more than 2 ants can kill a juvenile (Freidenfelds unpubl. data). In addition to immediate lethal effects, envenomation (via eating or attack) can cause delayed mortality (Langkilde & Freidenfelds, 2010). Populations of lizards from Red Imported Fire Ant-invaded sites have been shown to have behavioral and morphological traits that increase survival of fire ant attack (Langkilde 2009a; Robbins & Langkilde, 2012). This suggests that the Red Imported Fire Ant imposes novel selection on this species in the wild.

Since *S. invicta* venom can prove lethal to fence lizards, and encounters between these species are frequent, we expect lizards may adapt mechanisms to tolerate fire ant venom. The evolution of resistance to biological toxins and venoms has already been noted in several other squamates. Horned lizards have a detoxifying factor in their blood that allows them to eat their primary prey, venomous harvester ants (Schmidt, Sherbrooke, & Schmidt, 1989). The garter snakes *Thamnophis couchii* and *Thamnophis sirtalis* have developed elevated resistance to the neurotoxin tetrodotoxin, used for defense by their newt prey (Brodie et al. 2005; Brodie, Ridenhour, & Brodie, 2002). Additionally, two species of geckos in Israel have been shown to be

resistant to the effects of the venom of their scorpion prey (Zlotkin, Milman, Sion, & Werner, 2003).

Previous research suggests that Eastern Fence Lizards (*S. undulatus*) have not adapted to tolerate Red Imported Fire Ant venom. A comparison of Eastern Fence Lizards from a long-ago invaded population and an uninvaded population found no evidence of acquisition of physiological resistance of fence lizards to Red Imported Fire Ant venom as measured by effects of venom on whole-body performance and *in vitro* hemolysis, following fire ant invasion (Boronow & Langkilde, 2010). However, both populations of lizards tested in this study overlapped with the historical ranges of two native fire ant species, *Solenopsis xyloni* and *Solenopsis geminata*. This historical exposure to native fire ants may, therefore, have pre-adapted fence lizards to the venom of the invasive Red Imported Fire Ant, resulting in the fire ant-invaded and uninvaded lizards having equal venom resistance. We test this by comparing the venom tolerance of lizards from three *S. invicta*-uninvaded northern sites (designated UN) with no historical presence of fire ants, two *S. invicta*-uninvaded southern sites (designated US) with a historical presence of native *Solenopsis spp.*, and two *S. invicta*-invaded southern sites (designated IS) with a historical presence of native *Solenopsis spp*. Through this experimental design, we tested whether previous exposure of the Eastern Fence Lizard (*Sceloporus undulatus*) to native fire ants (*Solenopsis spp.*) has pre-adapted this lizard for physiological resistance to the venom of the Red Imported Fire Ant (*Solenopsis invicta*).

Chapter 2

Methods and Materials

Study Sites and Animal Collection

We collected 162 adult lizards from seven sites across the eastern United States which had similar elevations and habitat compositions, but differed in their fire ant invasion status. Seventy-nine lizards were collected from three uninvaded northern (UN) sites with no history of exposure to red imported fire ants (*S. invicta*) or other *Solenopsis spp.* (30 lizards at 39.896194N /74.57836W, New Jersey; 20 lizards at 40.367172N /79.23738W, Pennsylvania; and 29 lizards at 40.218867N/77.92159W, Pennsylvania). Forty lizards were collected from two uninvaded southern (US) sites with no history with *S. invicta*, but history with other native *Solenopsis spp.* (18 lizards at 36.0077842N/85.83238W, Tennessee; 22 lizards at 34.712056N/90.72549W, Arkansas). Forty-three lizards were collected from two invaded southern (IS) sites that were first invaded by *S. invicta* approximately 75 years ago and have history with other native *Solenopsis spp.* (20 lizards at 31.117169N/86.16783W, Alabama; 23 lizards at 31.164894N/86.70343W, Alabama).

For each lizard, sex and maturity were determined from the presence or absence of enlarged post-anal scales and abdominal and throat badges. Morphological data (mass, snout-to-vent-length; SVL) was also collected for each lizard. Lizards were group housed in the laboratory by site of capture with a maximum of 5 individuals per enclosure (60 X 42 X 30cm, L X W X D); groupings were organized so as to minimize the degree of male-male pairings to avoid aggressive interactions. Enclosures were lined with paper towels and furnished with a water bowl and a hide that served as a shelter and a perch site. Gravid females were housed in

enclosures with damp sand to allow for egg deposition and were returned to normal housing post-laying. An incandescent 60 W lamp was provided at one end of each enclosure to provide heat. Heat lamps and overhead fluorescent lighting were kept on a 12:12 hour cycle. Lizards were provided with *ad libitum* access to water and were fed live crickets every other day. The lizards' diets were supplemented weekly by dusting crickets with calcium (Rep-Cal Research Labs, Los Gatos, CA) and multi-vitamin powder (Sticky Tongue Farms, Sun City, CA). This study was conducted between the months of May and August, 2013 during peak activity of *S. undulatus*. All research was approved by the Institutional Animal Care and Use Committees at Penn State University (#35780) and Auburn University (#2011-1922).

Blood Lysis

We tested for blood lysis of fire ant venom following the methods of Boronow & Langkilde (2010). We obtained ~140μL of blood from the postorbital sinus of 76 lizards (11 lizards per site but one site with 10 lizards; 2 sites with six females and four males; 4 sites with five females and six males; 1 site with six females and four males; these lizards were a subset of those used for righting ability testing) using heparinized microhematocrit tubes. The blood was suspended in 300μL of Isotonic Blood Bank Saline (Nerl Buffered Saline, 20 liters from VWR, catalog #72060-034), shaken, and then centrifuged on a medium setting for 2 minutes. The erythrocytes were washed two more times with 300μL of saline and then re-suspended in 300μL of saline to form a "sample solution". We estimated the number of erythrocytes in the sample by taking 30μL of sample solution, diluting it in 40mL of saline in a p-cup, and counting erythrocytes in this aliquot with a Coulter counter (Z2 Dual Threshold, Beckman Coulter Inc., Fullerton, CA) with the following settings: 100μm aperture, K_d=59.07, Upper Threshold = 17, Lower Threshold = 9, and "count between". For the purposes of the experiment, it was assumed

that the sample counted was a homogeneous solution. Each sample was counted twice, with counts ranging from 20,000 to 40,000 erythrocytes; the count represents the number of erythrocytes in the portion of the solution taken up by the Coulter counter. The average of the count readings was used to calculate the volume of the "sample solution" which would contain 18,000 erythrocytes. This methodology allowed us to attempt to standardize the number of erythrocytes used in each hemolytic test. For each individual lizard tested, the standardized sample solution volume was added to each of four 1.5mL Eppendorf tubes. Enough treatment solution was then added to each tube so that the final volume of solution was 300μL for all tubes. The treatment solutions were: isotonic saline (zero lysis control), deionized water (high lysis control) and *S. invicta* venom alkaloid solution (experimental control treatment; two replicates). Each tube was shaken by hand for 1 minute and then incubated for 30 minutes at room temperature. After incubation, each tube was shaken for 1 minute and then centrifuged for 2 minutes on a medium setting. 200μL of supernatant was transferred directly to a 96-well microtiter plate and the absorbance was read at 440nm using a spectrophotometer (SpectraMax Plus, Molecular Devices, Sunnyvale, CA) within 30 minutes to determine changes in the color of the supernatant due to erythrocyte lysis (darker color indicates more lysis).

The venom alkaloids were obtained in crystalline form from R. Vander Meer (USDA-ARS, Gainesville, FL) and dissolved in saline to a concentration of 0.0595 g/L, a concentration which typically lysed between 50-70% of the sampled erythrocytes and allowed sufficient variation for analysis. Because venom alkaloids do not dissolve completely in saline, the venom solution was vortexed thoroughly prior to each use to ensure it was a homogeneous solution. Every lizard served as its own control, and the venom treatment was replicated twice for each subject (intra-assay coefficient of variation = 8.49%). To calculate the lysis of the erythrocytes (L), the following formula was used,

$$L = \frac{A_v - A_0}{A_{100} - A_0}$$

where A_v, A_0, and A_{100}, stand for the absorbance of venom, saline, and deionized water, respectively (Shin, Kang, & Hahm, 1999). A_v was calculated as the average of the two venom alkaloid replicates.

Whole Body Performance

The impact of *S. invicta* venom on whole body performance was tested following the methods of Boronow & Langkilde (2010). We used 162 lizards: 37 males and 42 females from uninvaded north (UN) sites, 19 males and 21 females from uninvaded south (US) sites, and 17 males and 26 females from invaded south (IS) sites. Whole body performance was measured as a lizard's righting ability, or the ability to flip back onto all four feet when placed on its back. The loss of a righting response is often used as the endpoint of endurance testing or toxicological studies and represents the point where the individual is no longer able to escape or injure a predator (Angilletta, Hill, & Robson, 2002; O'Donnell, Staniland, & Mason, 2007).

All testing was performed at the Solon Dixon Forest Research Center in Escambia County, Alabama. All lizards were tested on two consecutive days. On the first day of testing, we tested baseline performance in righting ability. Each lizard was removed from its enclosure and placed in a cloth bag for an hour prior to testing. After one hour, each lizard was removed from its bag and placed on its back in a paper towel lined container to begin the righting ability test; each lizard was tested three times consecutively. Each lizard's body temperature was measured using an infrared thermometer focused directly on the abdomen after the completion of the righting ability trial. On day 2, all lizards (except 23 control lizards) were exposed to natural envenomation by *S. invicta* prior to performance testing. Natural envenomation provides a more

realistic measure of the envenomation consequences that a lizard might face in the wild than could be achieved by artificial envenomation. Lizards (n = 139) were tethered with a cotton thread, placed on a disturbed fire ant mound, and exposed to attack by 4 to 20 ants (average = 8.1 ± 3.1 ants) for 5 to 125 sec (average = 42.0 ± 24.2 sec), following the methods of (Langkilde, 2009a). Any lizards that fled the mound during the envenomation protocol were replaced on the active mound to ensure a consistent degree of venom exposure. Venom exposure was quantified as the number of attacking ants times the duration of attack (ants*s) divided by the mass of each lizard (ants*s/g). Three observers were involved in the envenomation protocol. Twenty-three control lizards experienced similar handling, but were not exposed to fire ants, instead being placed on a patch of sand. Including the control lizards which were not exposed to natural envenomation, venom exposures ranged from 0 to 233 ant*sec/g (average = 33.7 ant*sec/g ± 35.9 (SD)). This range of doses was utilized because it mirrors a range of venom exposures from low to just sublethal, mimicking encounters with just a few foraging ants or encounters on or near an *S. invicta* mound. The upper limit of venom exposures used is near the lethal threshold for the eastern fence lizards (Langkilde, 2009a). Utilizing a range of exposures rather than a consistent dosage provides a better measure of resistance (e.g., Brodie & Brodie, 1990).

After envenomation, each lizard was placed in a cloth bag for an hour prior to performance testing to allow time for the lizard to begin metabolizing the venom. After a one hour incubation period, the lizard was tested for righting ability as on Day 1. All performance trials took place between the hours of 0800 and 1300 hours, and heat lamps were used to maintain lizard body temperatures at ~29°C during the trials.

Righting Ability

We used video footage to estimate righting ability, following the methods of Boronow &

Langkilde (2010). A video camera (Samsung SC-DC 73U DVD Camcorder, Samsung

Electronics Co. Ltd, China) was attached to a tripod and focused directly downward on the paper

towel lined container (60 X 42 X 30cm; L X W X D), used as the stage for the righting ability

testing. The lizard was placed on its back in the arena, directly below the camera. If the lizard

did not spontaneously flip over, it was gently prodded on its abdomen with a paintbrush to induce

the righting response. Immediately after righting itself, the lizard was again flipped back onto its

back until it had successfully righted itself three times. All of the righting trials were recorded

and the footage was later examined frame-by-frame in Windows Live Movie Maker (frame speed

of 1/30 sec per frame) to determine righting ability. The beginning of the right was defined as the

first frame in which one of the lizard's shoulders was elevated off of the ground, and the end of

the right was the first frame in which all four feet rested squarely on the ground. Therefore,

righting ability for this experiment is defined in seconds as 1/30 times the number of frames from

beginning to end, inclusive, required for the lizard to right itself. Shorter times indicated a faster

righting ability. The fastest righting time of all three replicates was used for statistical analysis.

Statistical Analysis

We used JMP (version 7, SAS Institute Inc., Cary, NC, USA) to analyze our resulting

data. Righting ability and venom exposure were log transformed and erythrocyte lysis was arcsin

transformed to fit the assumptions of the parametric tests. We tested venom tolerance indicated

by righting ability using ANCOVA with righting ability as the dependent variable, region (IS,

US, UN) and site nested within region as factors, and venom exposure (ants*s/g) as a covariate.

Sex, body temperature post-envenomation, mass, snout-to-vent length (SVL) and relative hind limb length (RHLL) were non-significant with respect to righting ability (sex: p = 0.0977; body temperature post envenomation: p = 0.6829; mass: p = 0.7077; SVL: p = 0.6738; RHLL: p = 0.7979). Therefore, these were excluded from the final model. We found no effect of observers in recording venom exposure (ant*s/g; $F_{2, 59} = 0.0909$, p = 0.9132) and this was also excluded from the final mode. Tukey's HSD was used to determine differences in righting ability among the three geographic regions tested. We tested venom tolerance indicated by hemolysis using ANOVA with erythrocyte lysis as a dependent variable, venom batch as a random effect, and region (IS, US, UN) and site nested within region as factors. Sex ($F_{1,63} = 0.8999$, p = 0.35) and sex*region ($F_{2, 63} = 2.1530$, p = 0.1246) were non-significant and were therefore excluded from the final model.

Chapter 3

Results

Righting ability differed between geographic regions (IS, US, UN; Figure 1; $F_{2, 152} = 9.36$, p = 0.0001; site within geographic region: $F_{4, 152} = 3.10$, p = 0.017). Post-hoc tests showed that the righting response of lizards from the uninvaded north (UN) was slower than that of the lizards from the uninvaded south (US) and invaded south (IS) populations, on average. Righting ability was not affected by venom exposure ($F_{1, 152} = 0.052$, p = 0.821), and this was true for all geographic regions (venom exposure*region: $F_{2, 152} = 1.73$, p = 0.181). Hemolysis did not significantly vary by geographic region (Figure 2; $F_{2, 66} = 0.033$, p = 0.9677; site within geographic region: $F_{4, 66} = 3.00$, p = 0.0243) or invasion status ($F_{2, 66} < 0.01$, p = 0.99).
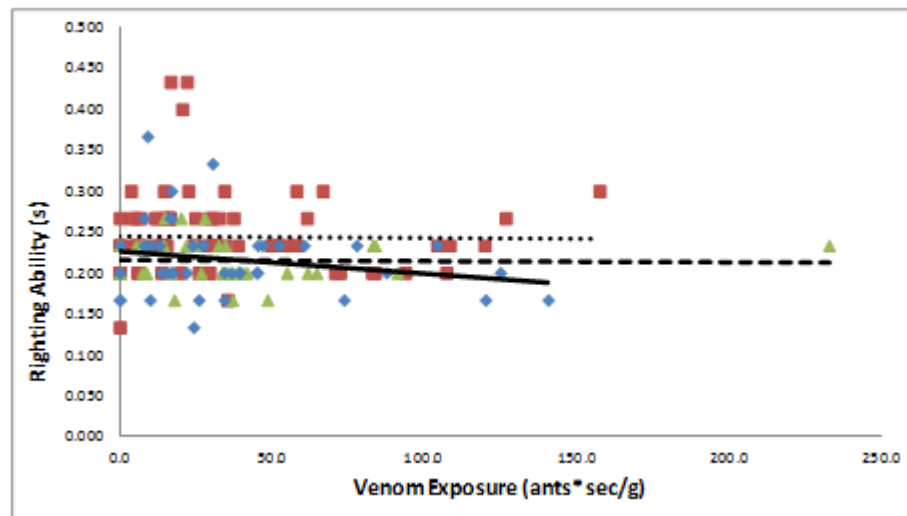
Figure 1: Effect of Venom Exposure on Righting Response

Time it took (in seconds) for lizards to right themselves after being placed on their backs, measured for fence lizards from two *S. invicta*-invaded sites (IS, blue diamonds, solid line, $R^2 = 0.0438$), from two southern uninvaded sites (US, green triangles, dashed line, $R^2 = 0.0004$), and from three northern uninvaded sites (UN, red squares, dotted line, $R^2 = 0.0002$). Lines represent best-fit regression lines. Each point represents a single individual.

Figure 2 Hemolytic Activity of Venom across Geographic Regions

The average percentage of fence lizards blood cells that are lysed by *S. invicta* venom, measured for lizards from three geographic regions; *S. invicta*-invaded south (IS), uninvaded south (US), and uninvaded north (UN). Error bars represent 1 standard error.

Chapter 4

Discussion

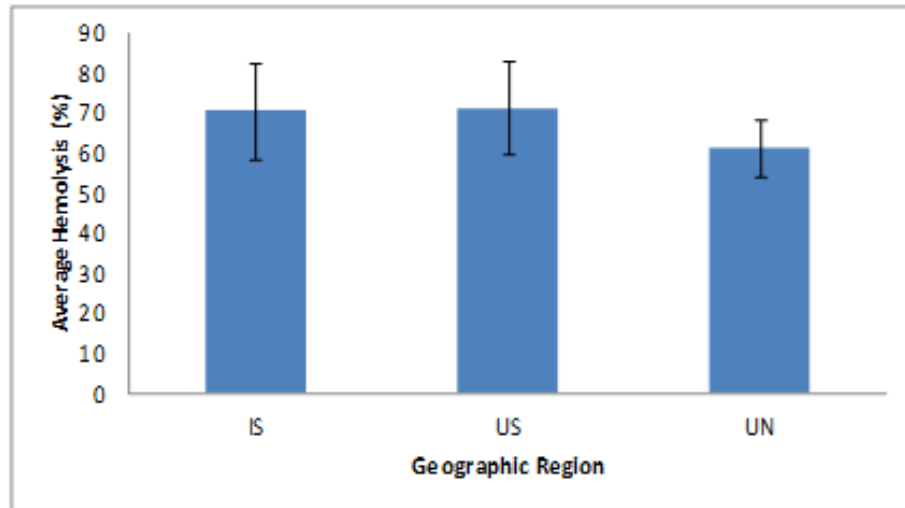We found no evidence that Eastern Fence Lizards are pre-adapted to Red Imported Fire Ant venom; populations with historical exposure to *S. geminata* and *S. xyloni* (IS: Alabama; US: Arkansas, Tennessee) did not have higher tolerance to *S. invicta* alkaloids than populations without history of exposure to native *Solenopsis spp.* (UN: Pennsylvania, New Jersey). There are several possible reasons for this. Although the native fire ants, *S. geminata* and *S. xyloni*, are closely related to the invasive fire ant, *S. invicta*, and their venom alkaloids share many components, these venoms are also quantitatively different (Brand, Blum, Fales, & MacConnell, 1972). So any adaptations these lizards may have to the native fire ants may not protect them from the invasive congener. It would be useful to test the hemolytic properties of *S. xyloni* and *S. geminata* venom on eastern fence lizard blood to determine if their venom is differently effective at subduing this native species.

Alternatively, the native fire ants may not have imposed a significant selection pressure for physiological adaptation to fire ant venom. The Red Imported Fire Ant (*S. invicta*) is more aggressive in nest defense and foraging than either *S. geminata* or *S. xyloni* (Morrison, 1999; Morrison, 2000), has stronger venom (Tschinkel, 2006), and occurs at higher densities than the native species (Morrison & Porter, 2004). The Red Imported Fire Ant can kill Eastern Fence Lizards (Langkilde, 2009a) as well as other vertebrates (Allen, Epperson, & Garmestani, 2004). *S. geminata* and *S. xyloni* can kill birds, but their capacity to kill lizards is largely unknown (Hooper-Bui, Rust, & Reierson, 2004; Travis, 1938). Behavioral trials similar to those done in Langkilde (2009a) could also be performed with *S. geminata* and *S. xyloni* colonies to determine

whether these ants will actively attack the eastern fence lizard. If the selective pressure imposed by these native fire ants was not as strong as that imposed by *S. invicta*, this would suggest that pre-adaptation to *S. invicta* is unlikely.

Our results also suggest that exposure to the invasive fire ant *S. invicta* has not selected for increased tolerance to venom (IS vs. UN). This is surprising since physiological resistance to venom generally evolves when the risk of envenomation is unavoidable, as when a predator is dependent upon toxic prey items (Brodie & Brodie, 1990). Eastern Fence Lizards from *S. invicta*-invaded sites actively incorporate *S. invicta* into their diets over native ant species (Robbins, Freidenfelds, & Langkilde, 2013) and can be stung in the mouth while feeding (Boronow & Langkilde, 2010). Although physiological resistance to venom has been shown in several other squamates (Brodie et al., 2005; Brodie, Ridenhour, & Brodie, 2002; Zlotkin, Milman, Sion, & Werner, 2003; Schmidt, Sherbrooke, & Schmidt, 1989), it does not appear to have evolved in this system. This is somewhat surprising given that fire ants appear to be imposing strong selection for adaptations to minimize the lethal and sublethal effects of *S. invicta* venom (Langkilde, 2009a). However, it appears that fence lizards may have other adaptations to modulate the amount of venom they receive. Eastern Fence Lizards from *S. invicta*-invaded sites have novel behavioral responses (body-twitch and flee response) and morphological traits (longer hind limbs increase the effectiveness of the behavioral response) that increase survival in the face of fire ant attack (Langkilde 2009a).

S. invicta fire ant alkaloids lysed an average of ~70% of erythrocytes for our *in vitro* experimentation. In fact, *S. invicta* alkaloids were more effective at lysing erythrocytes than was deionized water, which is typically used as a 100% lysis control (Ahmad, Perkins, Lupan, Selsted, & Janoff, 1995; Boronow & Langkilde, 2010).We did not, however, observe an effect of venom on righting ability suggesting that the venom exposures used in this experiment may not have been sufficient to induce impacts on whole-body performance. There appears to be a very

narrow threshold at which the venom goes from having no effect to paralyzing the lizard (Boronow and Langkilde, 2010), and the exposures we used were likely below this threshold.

While there were no differences in venom dosage-dependent righting ability across the geographic regions we tested, lizards from the uninvaded north sites (UN) were inherently slower in their righting response than those from southern populations (IS and US). This could be related to morphological or physiological differences in animals collected from these sites, such as musculature or baseline CORT levels (Goldy-Brown, unpubl. data) that could affect righting ability. While statistically different, it is unclear whether the difference in average righting response is of biological significance in the wild; would righting 0.03 seconds slower on average have significant fitness relevant implications? While the loss of righting response represents the point at which an organism can no longer escape or injure a predator (O'Donnell, Staniland, & Mason, 2007), it is possible that such a small decrease in righting response may not have such severe fitness consequences.

In conclusion, we found no physiological adaptation of native fence lizards to *S. invicta* venom. It is unlikely that this is due to pre-adaptation of these lizards to native fire ants as venom tolerance was similar among all geographical regions tested, even when testing lizards outside the range of native fire ant species. Given the likelihood of weak historical selection pressures imposed by native fire ants, this is not surprising. The role of pre-adaptation on impacts of invasive species should be considered in systems in which an invader has a close phylogenetic relationship to a native organism. This can help to elucidate whether any apparent lack of adaptation to an invader is actually a result of reduced selective pressure resulting from pre-adaptation to existing pressures. Knowledge of the potential role of pre-adaptation in species invasions could prove valuable to predicting and managing the impact of future invasions, which are likely to become more frequent as the globe becomes increasingly connected.

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Education:

B.S. in Biology (ecology option), May 2014
Schreyer Honors College   **Minor in Spanish**, May 2014
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Academics:

Undergraduate Research Assistant in the Langkilde Lab	Aug. 2012-Present
Undergraduate Research Assistant in the Marden Lab	Jun. 2012-Aug. 2012

Service:

Member of Springfield benefitting THON	Sept. 2010-Present

- 120 member organization raising over $200,000 annually

Relay for Life Participant	Apr. 2012, 2013
Service Trip Leader, *Schreyer Honors College Day of Service*	Mar. 2012, Mar. 2013
Schreyer Honors College Day of Service Participant	Mar. 2011
Fresh Start Day of Service Participant	Aug. 2010

Leadership:

Teaching Assistant for Biology 110 and Biology 110H	Su.2012, Aug. – Dec. 2013
Resident Assistant	Aug. 2012 – May 2013
Springfield Benefitting THON	
President	Mar.2013 – Present
4 Diamonds Recycling Effort Captain	Dec. 2012 - Feb. 2013
THON Weekend Captain	Jan. 2012 - Feb. 2012
Krew Leader	Fa.2011, Fa.2012 - Sp. 2013
Mentor, *Schreyer Honors College Orientation Program*	Jan. 2013 - Aug. 2013
Service Team Leader, *SHO TIME*	Jan. 2011 - Aug. 2011

Memberships/Awards:

Recipient of John W. Oswald Award for Scholarship	Mar. 2014
Member of The Honors Society of Phi Kappa Phi	Mar. 2014
Member of The Phi Beta Kappa Society	Mar. 2014
Dean's List for seven consecutive semesters	Fa.2010 - Present
Recipient of Summer Discovery Research Grant	Mar. 2013
Recipient of Undergraduate Research Grant ($300)	Nov. 2012
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