

THE NUMBER OF QUEENS IN RED IMPORTED  
FIRE ANT COLONIES AND TEXAS  
HORNED LIZARD DENSITY

by  
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## ABSTRACT

Invasive species, such as the Red Imported Fire Ant (*Solenopsis invicta*, hereafter, RIFA), can negatively impact native species via predation and modifying prey behavior. RIFA exist in two colony types, monogyne (single queen) and polygyne (multiple queens), and polygyne colonies are known to contain higher densities of fire ants than monogyne colonies. Texas horned lizard (*Phrynosoma cornutum*) eggs and hatchlings are suspected prey of RIFA's foraging and aggressive behaviors. In this study, we collected fire ants from Karnes City and Kenedy to determine if Texas horned lizard density is lower around polygyne colonies. We collected and sequenced 30 ants, of which 20 were RIFA. Counter to our expectations, there seemed to be no correlation between RIFA colony type and Texas horned lizard density. Furthermore, we found evidence that monogyne and polygyne colonies were coexisting. In future studies, we think larger sampling sizes and determining ratios of polygyne to monogyne colonies within the same area would be useful for further testing the hypothesis that colony type may affect horned lizard density.

## Introduction

Red imported fire ants (*Solenopsis invicta*) were introduced through the port of Mobile, Alabama, in the 1930s (Buren, 1972; Buren et al., 1974). They are native to South America where their populations are limited by competition with other ant species (Buren et al., 1974) and the presence of co-evolved predators (Porter et al., 1997). Outside of their native range, they usually become the dominant ant species due to their aggressive foraging behavior, high reproductive capability, and lack of predators and competitors (Allen et al., 2004). In the United States, two forms of red imported fire ant (hereafter 'RIFA') colonies exist, monogynous and polygynous (Greenberg et al., 1985; Porter et al., 1991). Monogynous colonies contain only one fertile queen and polygynous colonies contain multiple fertile queens. Densities of polygyne RIFA colonies tend to be three or more times larger than monogyne colonies (Macom and Porter, 1996).

The effects of RIFA on wildlife are well-documented (Allen et al., 2004). We chose to focus on the effects of RIFA on Texas horned lizards (*Phrynosoma cornutum*), specifically their effects on horned lizard prey, eggs, and hatchlings. Harvester ants (*Pogonomyrmex* spp.) are known to make up a significant portion of the Texas horned lizard diet. However, RIFA are competitively replacing harvester ants in their natural habitats (Wilson and Brown, 1958). In a laboratory setting, most harvester ant colonies were eliminated when a colony of RIFA was established nearby, and RIFA were observed attacking the harvester ants (Hook and Porter, 1990). It is worth noting that the RIFA used in these experiments are of the polygyne type (Hook and Porter, 1990). Therefore, it seems likely that the harvester ants, a staple in the Texas horned lizard diet, will decline in abundance in the presence of RIFA.

Texas horned lizards produce their offspring by laying eggs in a burrow about 20 cm underground (Sherbrooke 2003). The effects of RIFA on egg-laying reptiles are not well-documented, but a study by Diffie et al. showed that RIFA were able to enter the eggs of certain snake species, suggesting they might play a more prominent role in the decline of native reptilian species than previously thought (Diffie et al., 2010). RIFA also eat the young of ground nesting birds such as the Northern Bobwhite quail (*Colinus virginianus*) when they first break through the eggshell (Allen et al. 1995). Diffie et al., (2010) suggests that egg-laying species could be impacted because eggs are a possible food source for the ants. While their experiments focused on turtles, snakes, and avian species, we assume similar detrimental effects could occur with Texas horned lizard eggs if RIFA encounter them in the ground. If a Texas horned lizard manages to hatch from its egg, it may still face predation by RIFA. Unpublished results from reintroduction attempts with hatchling horned lizards have indicated that if hatchlings are released in areas with a lot of RIFA, they will be predated by the ants (Diane Barber, Fort Worth Zoo, pers. comm.). A study by Dziadzio et al. also showed that in gopher tortoises both eggs and hatchling survival was reduced in enclosures with RIFA, and survival rate was greater in enclosures with reduced numbers of fire ants (Dziadzio et al., 2016). Furthermore, their study suggested that exposure to RIFA resulted in behavioral changes in the hatchlings that can result in indirect survival challenges (Dziadzio et al., 2016). There may also be indirect effects such as RIFA decreasing small native species of ants the hatchlings depend on for food (Rachel Alenius, pers. comm.).

The decline of Texas horned lizards is multi-faceted, including habitat disturbance, pesticide use, the decline of their preferred food source, and possibly RIFA (Donaldson et al., 1994; Wolf et al., 2013). In south Texas, small towns still contain Texas horned lizards as well

as harvester ants (*Pogonomyrmex barbatus*) and RIFA. In this study, we asked whether RIFA colonies were monogyne or polygyne in two small towns in south Texas. We hypothesized that because of the increased densities of ants in polygyne colonies, Texas horned lizard densities at sites occupied by these types of colonies will be lower than lizard densities near monogyne colonies or that because there are horned lizards in these towns that colonies may predominately be monogyne.

## Methods

### **Field collection**

Texas horned lizards have been studied in the towns of Kenedy and Karnes City, Texas since 2013 at 15 sites (alley ways, school yards, parks, and abandoned lots) that are censused eight times each between June and August. These sites also contain varying numbers of harvester ant and RIFA colonies. Putative RIFA samples were collected in the summer of 2018 from eight of the 15 sites in these towns. RIFA colonies were located by their distinctive mounds and aggressive nature when disturbed and then ~10 ants were collected with forceps and placed in a 1.5 ml tube with 1 ml of 100% ethanol. GPS coordinates were taken for each sampled mound. Long-term averages of Texas horned lizards (5-9) years were calculated for each of the eight sites to compare to the RIFA colony types found at those sites. I compared the average number of Texas horned lizards at sites with different colony compositions and to the presence of an unidentified species using t-tests assuming unequal variances.

### **DNA extraction**

Five ants from a single mound were placed in 300  $\mu$ l of lysis buffer with 15  $\mu$ l Proteinase K (20 mg/ml). A drill and pestle were then used to grind up the ants and then the samples were

incubated overnight on a heating block at 55 °C. After cooling to room temperature, a half volume of 7.5 M Ammonium Acetate was added to each sample and then each sample was vortexed and placed in the freezer for 10 minutes. Samples were then centrifuged to pellet proteins and the resulting supernatant containing the DNA was mixed with 0.7 volumes of Isopropanol in a new tube. The samples were then centrifuged at full speed (16,000 rpm) for 20 minutes to pellet the DNA. Each pellet was washed with 400 µl of 70% ethanol, dried, and then resuspended in 75 µl of 10 mM Tris pH 8.0.

### **DNA Sequencing**

To determine if the samples were *S. invicta*, all samples were sequenced at a portion of the cytochrome oxidase I mitochondrial gene using the using universal primers dgLCO1490 (5'GGTCAACAAATCATAAAGAYATYGG 3') and dgHCO2198 (5'TAAACTTCAGGGTGACCAAARAAYCA 3') (Meyer 2003). PCR reactions were conducted in a 10 µl volume containing 1 µl of DNA, 1 µl of primer mix (dgLCO + dgHCO), 5 µl of Accutaq Supermix, 0.2 µl of BSA, and 2.8 µl of dH<sub>2</sub>O. PCR reactions were run for 1 cycle at 94 °C for 10 minutes, then 35 cycles at 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute, followed by 1 cycle at 72 °C for 5 minutes. 4 µl from each PCR product was separated on a 1% agarose gel and visualized to ensure amplification occurred.

ExoSap reactions to remove dNTPs and primers were run for 1 cycle at 37 °C for 15 minutes followed by 1 cycle at 80 °C for 15 minutes. Reactions were conducted in a 7 µl volume containing 5 µl of PCR product, 0.01 µl of ExoI, 0.2 µl of rSAP, 0.7 µl of 1X NEB 1 buffer, and 1.19 µl of dH<sub>2</sub>O.

The sequencing reaction was run for 35 cycles of 94 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 2 minutes. The reactions were conducted in 10 µl containing 1 µl of

ExoSap reaction product, 1  $\mu$ l of primer (either dgLCO or dgHCO), 1.75  $\mu$ l of 5X sequencing buffer, 0.5  $\mu$ l of BigDye, and 5.75  $\mu$ l of dH<sub>2</sub>O.

After sequencing, unincorporated dye-labeled nucleotides were removed by adding 20  $\mu$ l of BET to each reaction tube then the tubes were placed on a magnet. After magnetizing the tubes for about 20 seconds, the liquid was removed. The beads were cleaned twice with 100  $\mu$ l of 70% ethanol. The tubes were then removed from the magnet, and 40  $\mu$ l of 0.1 mM EDTA pH 8.0 were added to each reaction. The tubes were placed again on the magnet, and after about a minute, the liquid in each reaction tube was transferred to a semi-skirted plate. The plate was then run on the ABI 3130XL Genetic Analyzer. The resulting sequences were then compared to sequences in Gen Bank to determine if they were *Solenopsis invicta*. Only samples that had greater than 98% similarity to *Solenopsis invicta* sequences in the database were identified as *Solenopsis invicta*.

### **Monogyne vs polygyne identification**

The Gp-9 gene seems to be responsible for the existence of the two social forms of RIFA: monogyne and polygyne (Valles and Porter, 2003). Monogyne queens are homozygous, only possessing the Gp-9<sup>B</sup> allele, whereas polygyne queens are heterozygous, possessing Gp-9<sup>B</sup> and Gp-9<sup>b</sup> alleles (Ross and Keller, 1998). I amplified the Gp-9 region in all samples using Gp-9<sup>B</sup>-specific primers corresponded to positions 1683-1703 (26BS: 5'CTCGCCGATTCTAACGAAGGA) and 2167-2199 (16BAS: 5'ATGTATACTTTAAAGCATTTCCTAATATTTTGTC) and Gp-9<sup>b</sup> specific primers corresponding to positions 1307-1334 (24bS: 5'TGGAGCTGATTATGATGAAGAGAAAATA) and 1702-1729 (25bAS: 5'GCTGTTTTTAATTGCATTTCTTATGCAG) (Valles and Porter, 2003). PCR reactions were run for 1 cycle at 94 °C for 10 minutes, then 40 cycles at 94 °C for 30



seconds, 55 °C for 30 seconds, and 72 °C for 1 minute, followed by 1 cycle at 72 °C for 5 minutes. Reactions were conducted in a 20 µl volume containing 2 µl of DNA, 2 µl of primer mix (either 24BS and 16BAS or 24bS and 16bAS), 10 µl of Accutag, 0.4 µl of BSA, and 5.6 µl of dH<sub>2</sub>O. PCR products were separated on a 1% agarose gel and visualized (Fig. 1).

### Results

I successfully amplified and sequenced 30 of the 36 samples at the cytochrome oxidase I mitochondrial gene. Of the 30 fire ants sequenced, 20 were identified as *Solenopsis invicta* and 10 were identified as *Solenopsis* unidentified, suggesting the presence of another species at these sites (Table 1). Of the 20 *Solenopsis invicta* specimens, 11 (55%) were identified as polygyne due to amplification of the Gp-9<sup>b</sup> allele and 9 (45%) were identified as monogyne as the Gp-9<sup>B</sup> allele amplified but not the Gp-9<sup>b</sup> allele (Table 1). For the *Solenopsis* unidentified, amplification results were inconclusive for 8 of the 10 specimens and 2 resulted in monogynous identification.

There was no clear relationship between the average density of Texas horned lizards at a site and the type of RIFA colonies or the presence of the other *Solenopsis* spp. For example, both the lowest density (0.5 lizards per ha) and highest density (19.6 lizards per ha) sites had polygyne colonies and some sites had a mix of both colony types and species (Table 2, Fig. 2-5). I compared the average number of horned lizards at sites where only monogyne colonies were detected and sites where only polygyne or a mix of polygyne and monogyne colonies were found and there was not a significant difference ( $t = 2.45$ ,  $df = 6$ ,  $P = 0.65$ ). Similarly, there was not a difference in the average number of horned lizards and the presence of RIFA or the presence of RIFA and the other unknown species at a site ( $t = 2.78$ ,  $df = 4$ ,  $P = 0.10$ ) (Fig. 6).

**Table 1:** Location, DNA barcoding information, and colony type for each sampled ant colony

Colony ID #	Latitude	Longitude	Species	Match %	Colony type
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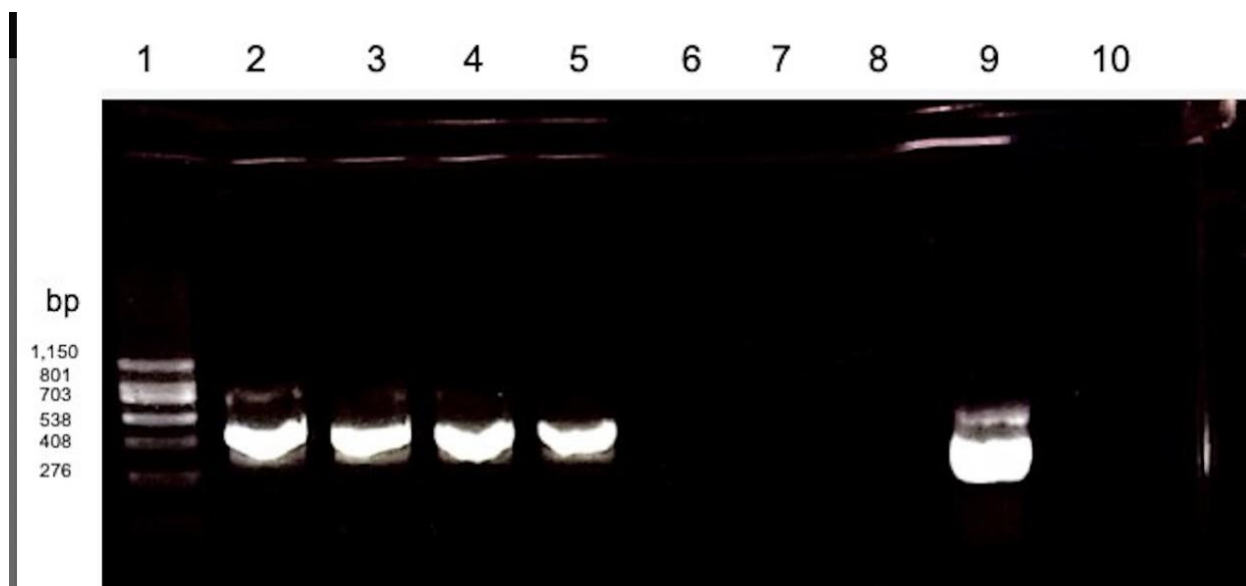
2	28.8091234	-97.847439	Solenopsis unidentified	99.2	--
3	28.8091474	-97.846837	Solenopsis invicta	99.4	Monogyne
4	28.809012	-97.846831	Solenopsis invicta	99.8	Monogyne
5	28.8091376	-97.84672	Solenopsis unidentified	98.9	Monogyne
6	28.8102706	-97.846013	Solenopsis invicta	99.8	Monogyne
7	28.810278	-97.846223	Solenopsis invicta	99.8	Polygyne
8	28.8877814	-97.899961	Solenopsis invicta	99.5	Polygyne
9	28.8873624	-97.899893	Solenopsis invicta	99.3	Polygyne
10	28.8876853	-97.903464	Solenopsis invicta	99.6	Monogyne
11	28.8875853	-97.903681	Solenopsis invicta	99.4	Monogyne
12	28.8880981	-97.905823	Solenopsis invicta	99.6	Monogyne
13	28.8977711	-97.901523	Solenopsis unidentified	99.6	--
14	28.8980352	-97.901492	Solenopsis invicta	98.9	Polygyne
16	28.8981313	-97.901454	Solenopsis invicta	99.3	Polygyne
17	28.8913843	-97.899121	Solenopsis invicta	99.8	Polygyne
18	28.8913825	-97.899174	Solenopsis invicta	99.8	Polygyne
20	28.8913471	-97.89941	Solenopsis unidentified	99.4	Monogyne
21	28.891423	-97.899507	Solenopsis unidentified	99.6	--
22	28.8912533	-97.899502	Solenopsis unidentified	99.5	--
23	28.8912678	-97.899244	Solenopsis unidentified	100	--
24	28.8911894	-97.89913	Solenopsis invicta	99.7	Monogyne
25	28.8911055	-97.899375	Solenopsis invicta	99.8	Polygyne
26	28.8911843	-97.899383	Solenopsis invicta	100	Polygyne

27	28.8911151	-97.899498	Solenopsis invicta	99.4	Monogyne
28	28.8911645	-97.899567	Solenopsis invicta	99.2	Polygyne
29	28.8910454	-97.899467	Solenopsis invicta	100	Monogyne
30	28.8910451	-97.898921	Solenopsis unidentified	100	--
33	28.8101023	-97.845625	Solenopsis invicta	99.6	Polygyne
34	28.8100628	-97.845563	Solenopsis unidentified	99.6	--
36	28.8100223	-97.845478	Solenopsis unidentified	98.1	--

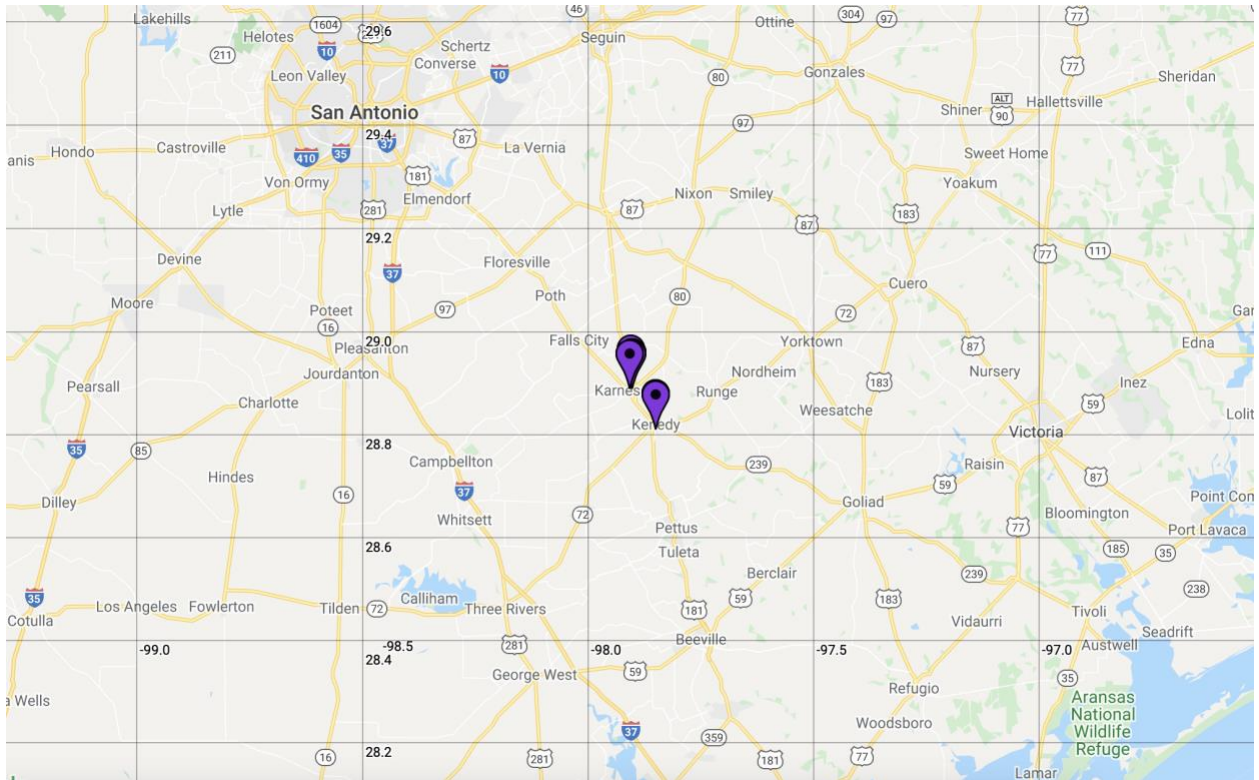
**Table 2:** Texas Horned Lizard densities at eight sites and their RIFA colony types

Location	Average number of Horned Lizards per ha	RIFA Samples	Colony Type
Tran	3.7	2, 3, 4, 5	M, Sspp
Barber	3.2	6, 7	M, P
Sch2	19.6	8, 9	P
Health Center	6.6	10, 11	M
Exercise	14.4	12	M
Soccer	1.3	13, 14, 16	P, Sspp
Water tower	5.3	17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30	M, P, Sspp
Brenda	0.5	33, 34, 36	P, Sspp

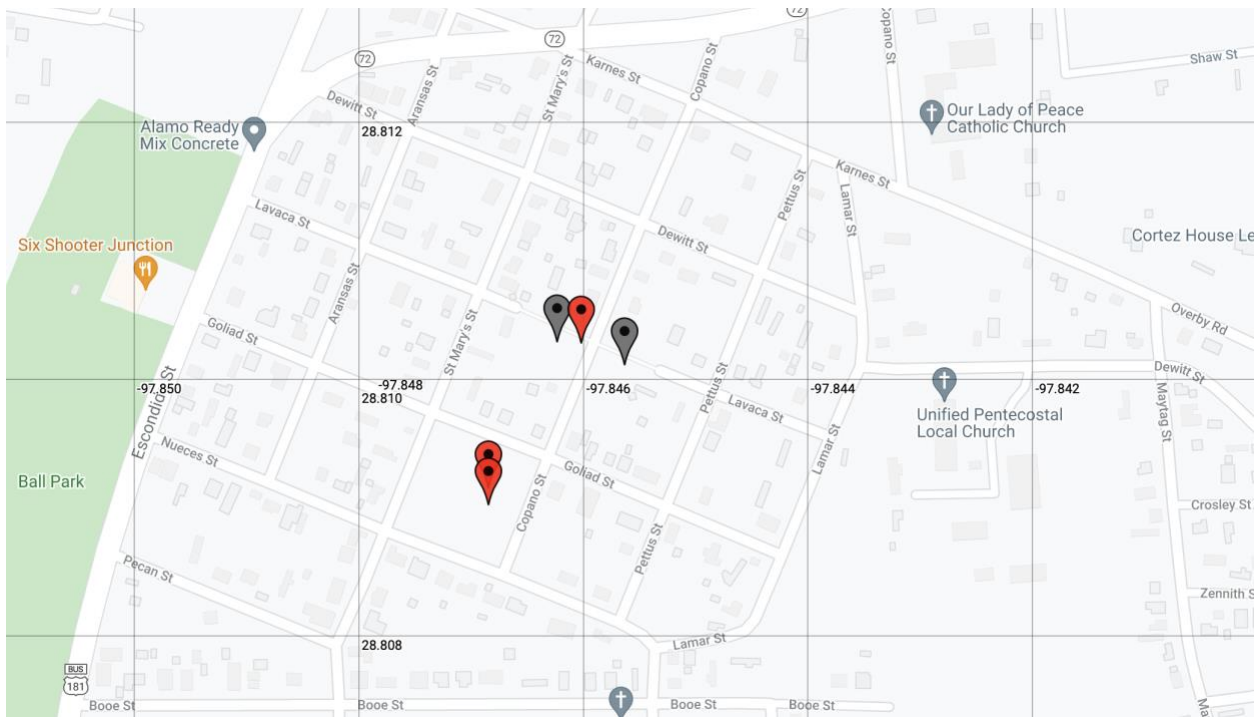
Table 2 Legend: P = Polygyne colony M = Monogyne colony Sspp = Solenopsis unidentified



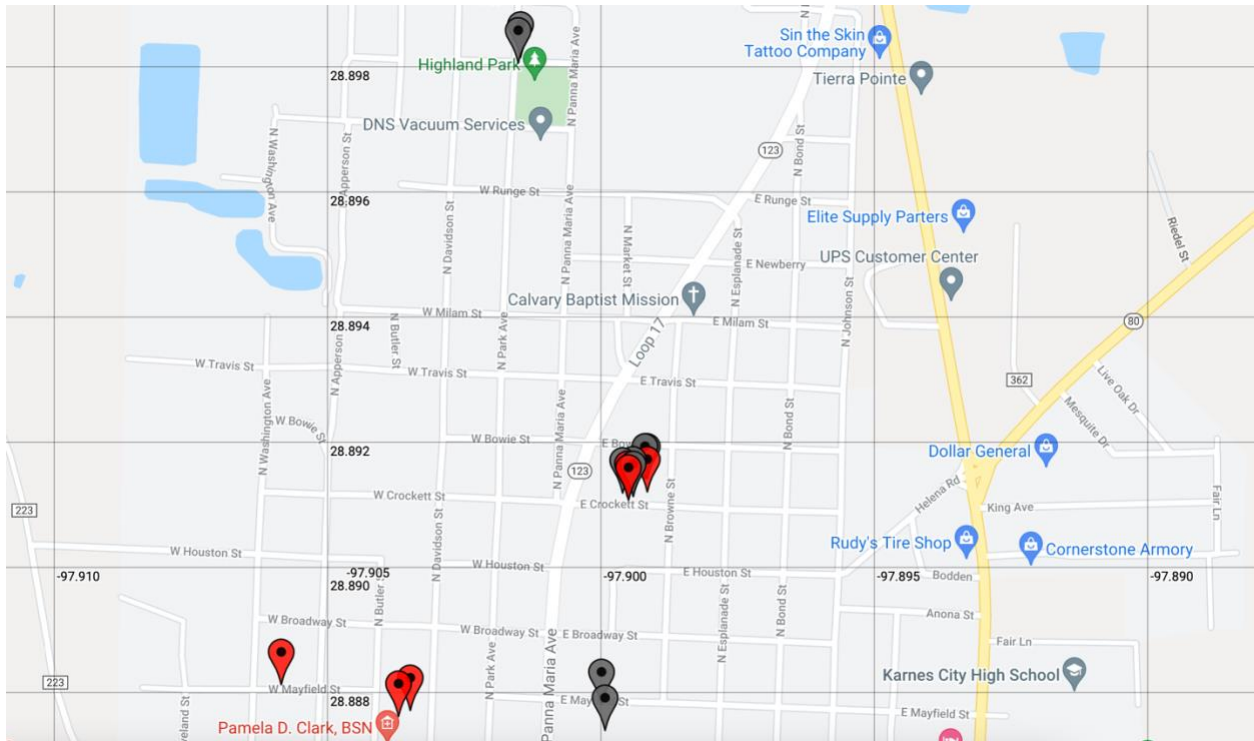
**Figure 1.** Banding patterns on a 1% agarose gel after multiplex PCR with Gp-9 allele-specific primers. Column 1, molecular weight markers expressed as base pairs; columns 2-5, PCR conducted with genomic DNA in the presence of Gp-9<sup>B</sup> specific primers; columns 6-9, PCR conducted with the same genomic DNA from columns 2-5, respectively, in the presence of Gp-9<sup>b</sup> specific primers. Columns 2-5 indicate that these genomic DNA samples belong to at least monogynous RIFA colonies. Column 9 indicates that this genomic DNA sample belongs to a polygynous RIFA colony.



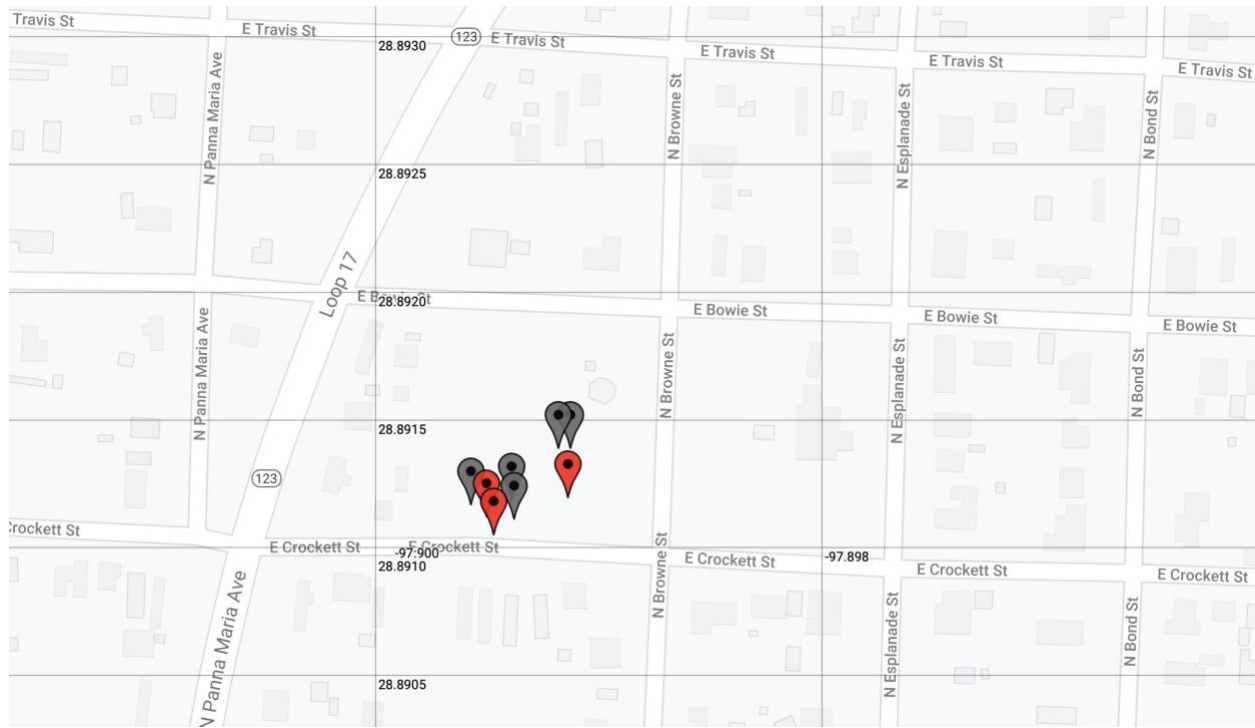
**Figure 2.** Purple pins indicate Kenedy and Karnes City where the putative RIFA samples were collected.



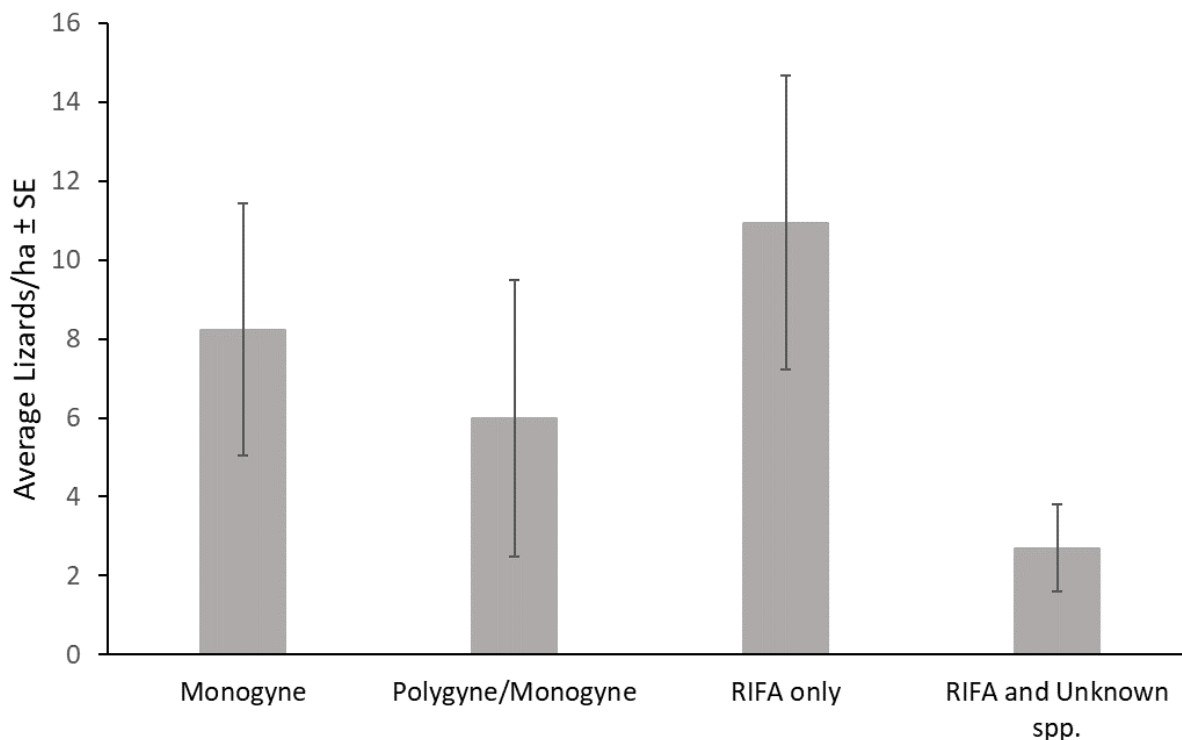
**Figure 3.** Samples identified as *Solenopsis invicta* collected in Kenedy marked by the pins. Red pins indicate monogynous colonies and grey pins indicate polygynous colonies.



**Figure 4.** Samples identified as *Solenopsis invicta* collected in Karnes City marked by the pins. Red pins indicate monogynous colonies and grey pins indicate polygynous colonies.



**Figure 5.** Zoomed-in view of samples identified as *Solenopsis invicta* collected in Karnes City between E Bowie St and E Crockett St marked by the pins. Red pins indicate monogynous colonies and grey pins indicate polygynous colonies.



**Figure 6.** Average density of Texas horned lizards in plots with single queen (monogyne) ( $n = 3$ ) or a mix of monogyne and multiple queen (polygyne) ( $n = 5$ ) red imported fire ant colonies (RIFA) and in plots where only RIFA were detected ( $n = 4$ ) and plots with a mix of RIFA and an unknown species of fire ant ( $n = 4$ ).

### Discussion

Counter to our expectations, the data suggests that there is no correlation between RIFA colony type and Texas horned lizard density. Furthermore, we were surprised to find monogyne and polygyne colonies existing amongst each other. Given the higher density of polygyne colonies, we thought polygyne colonies would displace monogyne colonies given the aggressive nature of RIFA. A study by Fritz and Vander Meer showed that polygyne colonies do not completely displace monogyne colonies, even after prolonged periods of time (Fritz and Vander



Meer, 2003). There is evidence that even after 13 or more years of polygyne presence in a certain part of Florida, monogyne colonies were still common (Fritz and Vander Meer, 2003). Reasons for these colonies coexisting appear to be due to reproductive limitations in exclusively polygyne areas. The production of male and female sexuals has been negatively correlated with queen number in *Solenopsis invicta*, so polygyne colonies produce fewer sexuals per unit time than those with single queens (Vargo and Fletcher, 1986; Vargo and Fletcher, 1987). Additionally, polygyne colonies mostly produce sterile males, so polygyne queens might depend on monogyne colony-derived males for successful mating (Hung et al., 1974; Ross and Fletcher, 1985; Fritz and Vander Meer, 2003). With our current sample size, the abundance of the two colony types appears to be similar in the regions we sampled. In future studies, we think sampling more sites with larger sample sizes of colonies so we can determine exact ratios of polygyne to monogyne colonies within sites may lead to more conclusive results.

In addition to RIFA, there were unidentified *Solenopsis* species present in the locations we sampled. There was also no difference in horned lizard density between plots that contained just RIFA or sites that also had this unidentified species, although there may have been a trend ( $P = 0.10$ ) for horned lizards to be more abundant at sites with just RIFA. Larger sample sizes across more sites would be useful to test this trend further. In Texas, there are two native fire ants, *Solenopsis geminata* and *Solenopsis richteri*, both of which are sequenced in Gen Bank. However, none of the ten unidentified *Solenopsis* species had sequences similar enough to either of these two native species to be considered one of them. Therefore, these results need to be researched further to determine what species these ants are, as fire ant species are difficult to identify by morphology alone.

#### References

- Allen, C. R., D. M. Epperson and A. S. Garmestani. 2004. Red imported fire ant impacts on wildlife: a decade of research. *The American Midland Naturalist.*, 152(1):88-103.
- Allen, C.R., R.S. Lutz and S. Demarais. 1995. Red imported fire ant impacts on northern bobwhite populations. *Ecol. Appl.* 5(3):632-638.
- Buren, W. F. 1972. Revisionary studies on the taxonomy of the imported fire ants. *J. Ga. Entomol. Soc.*, 7:1–26.
- Buren, W.F., G. E. Allen, W. H. Whitcomb, F. E. Lennartz and R. N. Williams. 1974. Zoogeography of the imported fire ants. *J. NY Entomol. Soc.*, 82:113–124.
- Diffie, S., J. Miller and K. Murray. 2010. Laboratory observations of red imported fire ant (Hymenoptera: Formicidae) predation on reptilian and avian eggs. *J. Herp.*, 44(2):294-296.
- Donaldson, W., A. H. Price, and J. Morse. 1994. The current status and future prospects of the Texas horned lizard (*Phrynosoma cornutum*) in Texas. *Texas J. Sci.*, 46:97-113.
- Dziadzio, M. C., R. B. Chandler, L. L. Smith and S. B. Castleberry. 2016. Impacts of red imported fire ants (*Solenopsis invicta*) on nestling and hatchling gopher tortoises (*Gopherus polyphemus*) in Southwest Georgia, USA. *Herp. Conser. Biol.*, 11:527-538.
- Fritz, G. N. and R. K. Vander Meer. 2003. Sympatry of polygyne and monogyne colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.*, 96(1):86-92.
- Greenberg, L., D. J. C. Fletcher and S. B. Vinson. 1985. Differences in worker size and mound distribution in monogynous and polygynous colonies of fire ant *Solenopsis invicta*. *J. Kansas Entomol. Soc.*, 58:9–18.
- Hook, A. W. and S. D. Porter. 1990. Destruction of harvester ant colonies by invading fire ants

- in South-Central Texas (Hymenoptera: Formicidae). *Southwestern Nat.*, 35(4):477-478.
- Hung, A.C.F., S. B. Vinson, and J. W. Summerlin. 1974. Male sterility in the red imported fire ant, *Solenopsis invicta*. *Ann. Entomol. Soc. Am*, 67:909-912.
- Macom, T.E. and S. D. Porter. 1996. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. *Ann. Entomol. Soc. Amer*, 89:535-543.
- Meyer, CP. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* 79: 401–459.
- Porter, S. D., A. Bhatkar, R. Mulder, S. B. Vinson and D. J. Clair. 1991. Distribution and density of polygyne fire ants (Hymenoptera: Formicidae) in Texas. *Econ. Entomol.*, 84:867–874.
- Porter, S. D., D. F. Williams, R. S. Patterson and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies. *Environ. Entomol.*, 26:373–384.
- Ross, K.G. and D. J. C. Fletcher. 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. *Evolution*, 39:888-903.
- Ross, K.G. and L. Keller. 1998. Genetic control of social organization in an ant. *Proc. Natl. Acad. Sci. USA*, 95:14232–14237.
- Sherbrooke WC. 2003. Introduction to horned lizards of North America. Berkeley: University of California Press.
- Valles, S. and S. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of Gp-9 alleles. *Insectes soc.*, 50:199-200.

- Vargo, E. L., and D. J. C. Fletcher. 1986. Evidence of pheromonal queen control over the production of male and female sexuals in the fire ant, *Solenopsis invicta*. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 159:741-749.
- Vargo, E. L., and D. J. C. Fletcher. 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. *Physiol. Entomol.* 12:109-116.
- Wilson, E. O., and W. L. Brown, Jr. 1958. Recent changes in the introduced population of the fire ant *Solenopsis saevissima* (Fr. Smith). *Evolution*, 12:211-218.
- Wolf, AJ, EC Hellgren, V Bogosian III, and RW Moody. 2013. Effects of habitat disturbance on Texas horned lizards: an urban case study. *Herpetologica*, 69(3):265-281.