A Biodiversity Survey of Ants (Hymenoptera: Formicidae) in College Station, Texas

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Abstract: Biodiversity of any given area is important to assess which species are present, what invasive species are disrupting the ecosystem, and how they interact with and affect the native species. By knowing what species exist, conservation efforts can be taken underway to conserve the native species or rid the invasive species. This research is a biodiversity survey of three different parks within College Station, TX. Each park had a variety of meals (protein, lipid, carbohydrate, and sugar meal) that was available to ants for 45 minutes to attract as many species as possible. Ants were identified on the scene as to not disrupt their recruitment process. The ants identified were *Solenopsis invicta* (Hymenoptera: Formicidae, Buren), *Monomorium pharaonis* (Hymenoptera, Formicidae, L.), *Monomorium minimum* (Hymenoptera, Formicidae, Buckley), and *Nylanderia fulva* (Hymenoptera, Formicidae, Lennon). Of the four species found two were native (*M. pharaonis* and *M. minimum*) and two were invasive (*S. invicta* and *N. fulva*). Red imported fire ants outcompeted all other ants and showed significantly higher numbers than the other species observed.

Keywords: biodiversity, invasive, Monomorium minimum, Monomorium pharaonis, native, Nylanderia fulva, Solenopsis invicta

Biodiversity surveys can give insight to what organisms are present, how they interact, and the implications of invasive species with native species (Lõhmus et al. 2018). One thing can be accomplished through a biodiversity survey of any given area is conservation efforts that might need to be taken under way to conserve the native species (Tanalgo et al. 2019).

Native and Invasive Species. The native species of ants that exist in College Station, TX are the pyramid ant (*Dorymyrmex insanus*, Buckley), the bigheaded ant (*Pheidole megacephala*, F.), the acrobat ant (*Crematogaster spp.*, Lund), the odorous house ant (*Tapinoma sessile*, Say), and the little black ant (*Monomorium minimum*, Buckley) (Caprio et al. 2017). Of these known native species, only *M. minimum* was observed (Figure 1). Another species that was observed was the Pharoah ant (*Monomorium pharaonis*, L.) which is also native to the area (Wetterer 2010).

The invasive species that are commonly found in College Station, TX include the Argentine ant (*Iridomyrmex humilus*, Mayr), the red imported fire ant (*Solenopsis invicta*, Buren), the tawny crazy ant (*Nylanderia fulva*, Lennon), and the ghost ant (*Tapinoma melanocephalum*, F.) (Caprio et al. 2017). Of

these invasive species, only the red imported fire ant and the tawny crazy ant were observed (Figure 1).

Invasive Nature of the Red Imported Fire Ant. Since the introduction of the red imported fire ant, *S. invicta*, in the early 1930s (Morrison et al. 2004, Vinson 1997), they have moved westward through Texas at a rate of 198 kilometers per year; northern movement has been slowed significantly because of the colder climate (Vinson 1997). Since these are an invasive species, they are no doubt going to have an impact on local agriculture, home life, and the native species that exist there (Jetter et al. 2002). In 1997, *S. invicta* were discovered in California; they have disrupted agricultural harvests because of their aggressive nature anywhere from $12 million at low infestation levels, to $30 million at high infestation levels (Jetter et al. 2002). The average household has been spending money on yard treatments and damage repair totaling anywhere from $33 (low-risk area) to $104 (high-risk area), with $80 being the average total annual cost per household (Jetter et al. 2002).

One study has shown that there is a negative correlation between the presence of *S. invicta* and species diversity and richness in Mississippi (Epperson and Allen 2010). Another study has shown that the competition of *S. invicta* can reduce species diversity when introduced to an undisturbed habitat (LeBrun et al. 2012). Following this, a more recent study showed that invertebrate and vertebrate levels had returned to normal levels pre-*S. invicta* invasion in Texas (Sawyer 2019) indicating that the environment in which they exist in has reached some version of equilibrium.

Invasive Nature of the Tawny Crazy Ant. Another other invasive ant species found in Texas was *N. fulva*, otherwise known as the tawny crazy ant or the raspberry crazy ant (Wang et al. 2016). They originated from South America just like *S. invicta* did (Ridlon et al. 2021) and were first seen in Houston, TX in 2002 (MacGown and Layton 2009). Their invasive nature is like *S. invicta* where they displace the native species as well as annoy homeowners and farmers (Wang et al. 2016, Chen et al. 2013); however, this ant is so invasive that it has gained extensive media coverage due to its ability to outcompete the invasivity of *S. invicta* (Gotzek et al. 2012, Meyers 2008).

Since their discovery in 2002, they are prevalent in two main counties: Harris County and Brazos County (MacGown and Layton 2009). Tawny crazy ants (*N. fulva*) are much more aggressive than the red imported fire ants that are already invasive—so much more that they have been used as a biological control agent in other areas (Chen et al. 2013). The reason they have been used a s a biological control agent is because of their defensive secretions. They squirt two major components from their Dufour glands: undecane and 2-tridecanone (Chen et al. 2013), that are primarily used as a toxin or repellant.

To gain a broad scope of the biodiversity of any given area, selecting locations that are going to be representative of the entire area is crucial. Three locations that were relatively

diverse in the makeup of the surrounding environment was chosen. Three parks all exhibiting different environments or microclimates that are roughly two hectares (Lõhmus et al. 2018). To attract a variety of ants, a variety of diets were used. Ants have no specific preference on diet choice, but they had the option (Ridlon et al. 2021). After a survey is complete, the native and invasive species can be identified to understand their relationship with one another

Materials and Methods

Location. Three locations were scouted out with ant mounds to survey the biodiversity of ants in the area. Each location was in a park in College Station, TX, and each park was far away from each other. Each park surveyed also showed similar levels of human interaction.

Attraction and Identification. To attract as many different ant species as possible to the locations, a variety of nutrient-rich meals (protein, lipid, carbohydrate, and sugar) were placed on the ground to attract the ants. To prepare the protein meal, ~40g of pistachios (HEB, San Antonio, TX) were allowed to soak in water for roughly 15 minutes to soften them. The same was done for the lipid meal with almonds (~40g) (HEB, San Antonio, TX). The carbohydrate meal was bread (~20g) (HEB, San Antonio, TX) that was lightly spritzed with water to make it moist and a softer for the ants to eat. The sugary meal was prepared by taking 25g of sugar (Imperial Sugar, Sugarland, TX) and mixing it with enough water (~25mL) to form a paste-like substance. Once the meals were prepared, they were placed within three inches of each other on the ground. The meals were left undisturbed for 45 minutes. Every five minutes a photo was taken noting the number of ants and the species. The species of the ants were identified on site using a dichotomous key found online (Mueller lab). After 45 minutes passed with the meals sitting out, the final photo was taken, and the meals were removed. This was repeated once at each of the three locations.

Calculating the Biodiversity. After all counts and species identification had been obtained, the biodiversity index was calculated for each ant species observed. This was calculated by taking the number of a specific species and dividing it by the number of total ants observed; this was calculated for each site as well as the entire sample size. The biodiversity index is representative of the percent of ants observed. A one-way ANOVA with post hoc Tukey HSD was calculated to determine if there was statistical difference between the amount of each ant species observed and if the number of total ants observed between locations was significantly different.

Results

A total of four ant species were attracted. These included *S. invicta*, *M. minimum*, *M. pharaonis*, and *N. fulva*. The total number of each ant was recorded at each five-minute time interval (Table 1).

Through the one-way ANOVA analysis, the *p-value* between the locations was found to be $p=0.8374$ (M=96.66, SD= 35.73). This indicates that there was not a significant difference between the number of ants that

were observed between each location. The *p-value* between *S. invicta* (M=78.33, SD=38.70), *M. minimum* (M=0.33, SD=0.58), *M. pharaonis* (M=15, SD=25.98), and *N. fulva* (M=3, SD=5.20) was calculated to be *p=0.0110*. This indicates that there was a significant difference between the number of each species that was observed. To determine the relatedness of each species, a Tukey HSD post-hoc test was run. Figure 1 shows the results of the Tukey-post hoc test and that there was a statistical difference between these four species. There was statistical difference between the number of *S. invicta* compared to *M. minimum*, *M. pharaonis*, and *N. fulva*. The only meal that the ants were attracted to was the sugar meal; no ants were observed on the other meals.

The biodiversity index of *S. invicta* was found to be 0.8319, for *M. minimum* it was 0.0060, for *M. pharaonis* it was found to be 0.1351, and for *N. fulva* it was found to be 0.0270.

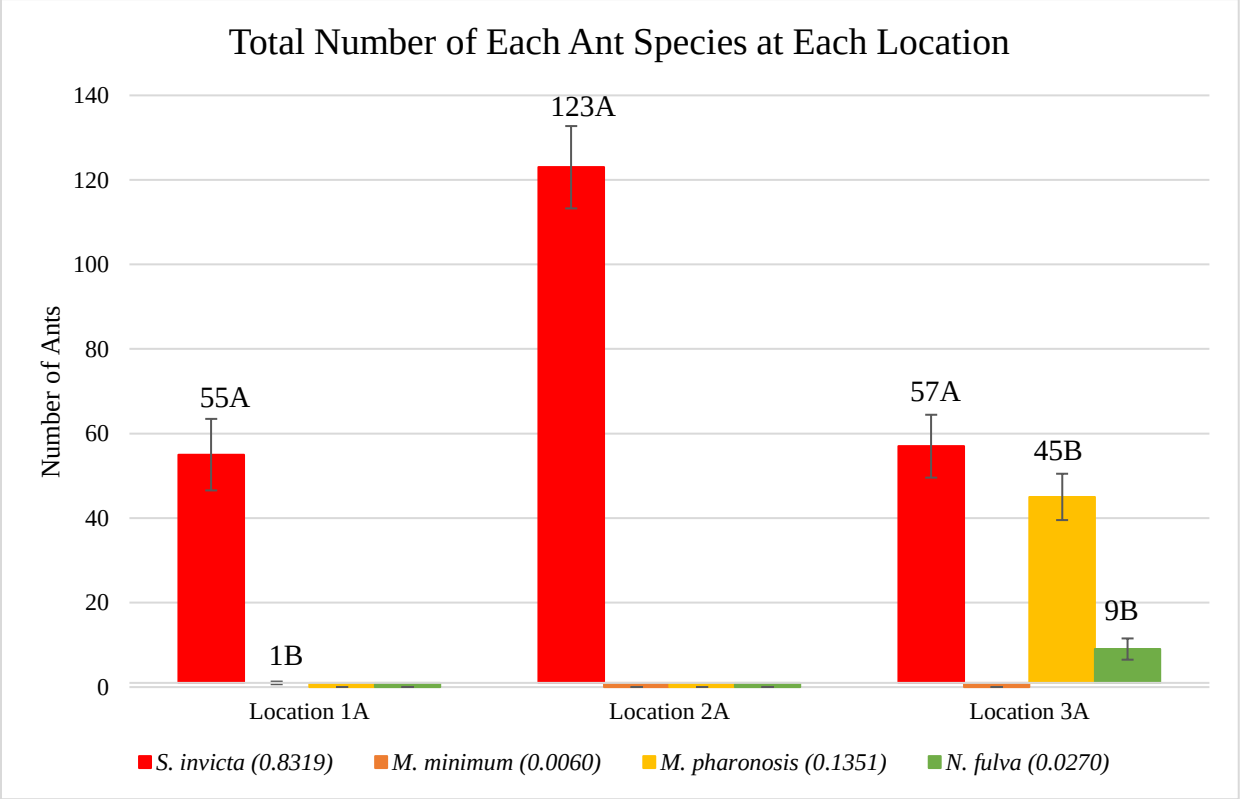

Figure 1. Letters such as "A" or "B" next to the number of observed ants represent statistical difference. If there is statistical difference ($p<0.05$), a Tukey HSD post-hoc test was run. Different letters represent statistical difference, same letters represent no statistical difference ($p>0.05$). The "between locations" *p-value* = 0.8374 (not statistically different). The "between species" *p-value* = 0.0110 (statistically different). Tukey HSD yielded that there was significantly more *S. invicta* (A) than the other three species observed (B). Number in parenthesis next to the labels represent the biodiversity index.

Table 1. The total number of ants, separated into species, recorded at each location. Mean and standard deviation are for each time interval, not the total amount of ants observed. "L" stands for location. Letters (A or B) next to location or species indicates statistical difference. There was no statistical difference between the number of ants observed at each location (p=0.8374).

Number and Location of each Ant Species					
Location (L) #	Time (min)	*Solenopsis invicta* (A)	*Monomorium minimum* (B)	*Monomorium pharaonis* (B)	*Nylanderia fulva* (B)
Location 1 (A) (low interaction)	0	0	0	0	0
	5	0	0	0	0
	10	0	0	0	0
	15	2	0	0	0
	20	2	0	0	0
	25	2	1	0	0
	30	3	0	0	0
	35	5	0	0	0
	40	15	0	0	0
	45	26	0	0	0
Mean		5.5	0.1	-	-
Standard Dev.		8.46	0.32	-	-
Location 1 total		55	1	0	0
Location 2 (A) (high interaction)	0	0	0	0	0
	5	1	0	0	0
	10	30	0	0	0
	15	23	0	0	0
	20	3	0	0	0
	25	19	0	0	0
	30	15	0	0	0
	35	11	0	0	0
	40	10	0	0	0
	45	11	0	0	0
Mean		12.3	-	-	-
Standard. Dev		9.74	-	-	-
Location 2 total		123	0	0	0
Location 3 (A) (medium interaction)	0	0	0	0	0
	5	0	0	0	0
	10	1	0	1	0
	15	4	0	9	0
	20	2	0	15	1
	25	2	0	10	0
	30	2	0	8	0
	35	8	0	1	8
	40	21	0	0	0
	45	17	0	1	0
Mean		5.7	-	4.5	0.9
Standard Dev.		7.44	-	5.48	2.51
Location 3 total		57	0	45	9
Total (L1, L2, L3) Ants Observed		235	1	45	9

Discussion

The main finding of the biodiversity survey was that *S. invicta* dominated out of the three other ant species observed: *M. minimum, N. fulva*, and *M. pharaonis*. The three regions surveyed showed that there was no difference in number of ants found at each location—meaning all locations had similar ant activity.

Red Imported Fire Ant: *S. invicta.* The red imported fire ant was the main ant observed in all three locations, dominating the other

three species found. This aligns with previous work done to assess the biodiversity of areas in Texas (Claborn and Philips 1986, Cockendolpher and Philips 1989, Caprio et al. 2017). Since 1957, *S. invicta* have been expanding across Texas (Cockendolpher and Philips 1989). This data collected aligns with other studies that show *S. invicta* being distributed in large densities across Texas. Caprio et al. (2010) found that *S. invicta* dominated three out of four locations surveyed in College Station, TX. This would explain their high biodiversity index of 0.8317 (Figure 1).

Little Black Ant: *M. minimum.* The little black ant was also found in College Station, TX by a similar study (Caprio et al. 2017). Since this species is a native species, it is competing with the invasive species that are in the area. This would explain the low biodiversity index found for this ant species at 0.002 (Figure 1).

Pharaoh Ants: *M. Pharaonis*. Finding this ant was somewhat of a surprise, but it also lines up with previously published material. According to Wetterer (2010), this species of ant is commonly found in around 25% of hospitals in Texas. The pharaoh ant, *M. pharaonis,* was found to have a biodiversity index of 0.1351 (Figure 1).

Tawny Crazy Ants: *N. fulva.* The tawny crazy ant, *N. fulva,* was discovered during this survey and was found to have a biodiversity index of 0.0270 (Figure 1). Comparing to other studies, this ant has been found in its "normal" geographic range since its discovery in Texas in 2002 (Eyer et al., 2018). Eyer also found that this ant was present in Bryan, TX, a neighboring city to College Station, TX. Through further research, this ant was found to be invasive from South America just like *S. invicta* (MacGown and Layton 2009).

Competition. Another thing to consider is the competition between the invasive species and the native species. This depends on whether the colony is polygyne, which means more than one queen, or monogyne, which means one queen only. The impacts of polygyne colonies are more ecologically relevant; however, most fire ant colonies are found to be monogyne (LeBrun et al. 2012) which reduces their invasivity and competition with native species. When analyzing the competition of the fire ants, higher density indicates higher competition (LeBrun et al. 2007).

One limitation is the short sample time and smaller sampling technique. This can be improved by sampling more locations, with more samples observed in each location. Another limitation of this research was the short time frame the data was collected in. If the research was more spread out like in the study done by Cockendolpher and Philips in 1986 and 1989, there would have been more accurate data. Another problem, later identified after the experiment was conducted, was that since ants have no preference for diet (Ridlon et al. 2021), a sugar water mix could have been used to have the strongest attraction. Most ants found were on the sugar meal than any other meal.

This research reinforced what is already known—that fire ants (*S. invicta*) dominate this region and outcompete all other ants; however, another thing that was discovered through more research was that the tawny crazy ants (*N. fulva*) are an invasive species as well that competes heavily with *S. invicta*. Because they are an invasive species, the already existing native ant species have not evolved to defend themselves against the invasive species. Claborn and Philips (1986) found that when other ant species are foraging with fire ants present, there are significantly lower numbers of the other ant species observed. This was reflected in the data collected (Figure 1, Table 1).

This research could be used in the future to try and conserve the native ant species that are found in College Station, TX as well as research methods to rid the invasive species. This could also be used to understand each organism's unique role in the ecosystem in which they inhabit. These results might be used in the real world when simply going outside and trying to rid your lawn of the invasive red imported fire ant. Looking for poison that targets them but not the native species would be beneficial to the environment. This data could also be used to attempt and identify the ants observed in different locations.

Overall, the red imported fire ant was the most common species of ant observed out of the three other species observed: the little black ant, the pharaoh ant, and tawny crazy ant. When walking out of your home, you are most likely to see fire ants (*S. invicta*) rather than any other ant species. This is due to their invasive nature.

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