

A STUDY OF CENTRAL TEXAS *NYLANDERIA FULVA* (HYMENOPTERA: FORMICIDAE)  
AGGRESSION WITH COMPETING ANT POPULATIONS AND DIET PERFORMANCE

A Thesis

by

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## ABSTRACT

*Nylanderia fulva*, native to South America, is an ecologically dominant invasive ant in the U.S. that has spread to central Texas. This species is able to quickly form large colonies and displace established ant populations in its invasive range, reducing local ant biodiversity. Because populations of *N. fulva* have only recently begun to expand rapidly there is much we do not know about this ecologically and economically important species. The studies presented here were aimed to better understand *N. fulva*'s remarkable displacement of local ant populations, as well as provide an effective method of rearing *N. fulva* long term, so that important research may be carried out expeditiously.

In order to help elucidate how *N. fulva* is able to displace local ant populations, aggression assays were carried out between *N. fulva* and common ant species present in central Texas. These results indicate that *N. fulva* is highly aggressive to both *Solenopsis invicta* and larger bodied ant species. This matches field observations of ant displacement due to *N. fulva* invasion, indicating that *N. fulva* may displace local ant species through direct confrontation.

*N. fulva* populations become scarce during the winter, and do not begin to resurge until late spring, making collections and research difficult during this time. It is also difficult to keep *N. fulva* colonies in a lab setting. In order to ameliorate this, a diet performance study was performed so that stock colonies of *N. fulva* may be kept over a long period of time, so that research may continue on this invasive ant throughout the year. Of all diets we tested, our results show a diet of the beet armyworm *Spodoptera exigua* augmented with an artificial insect diet resulted in the highest level of brood production and colony growth over the course of the study.

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Finally, thanks to my mother and father for their encouragement and to my fiancé for her understanding, love, and her unfailing ability to put a smile on my face.

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# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

Ants are present in many diverse habitats throughout most of the world. The only places on earth without native ant species are Greenland, Antarctica, and remote islands in the Pacific and Indian Oceans (Hölldobler and Wilson, 1990). Where ants do occur, they perform many important functions in their respective habitats. For example, ants can either match or exceed earthworms in aerating soil, and are among the top seed dispersers in their environments, even rivaling mammals in their importance in dispersing seeds (Lyford 1963, Handel et al. 1981). There are roughly 14,000 species of ants which have been described (Agosti & Johnson 2005). The vast majority of these ants are beneficial species, providing important ecological services such as the ones listed above. However, a small fraction of these species are invasive. These invasive species occur outside their native range and often have major ecological and/or economic impacts where they have been introduced.

Although ants perform many important ecological services, an invasive ant species can greatly disrupt the ecology of the introduced range. As mentioned above, ants are important seed dispersers. However this only applies to native ants. Invasive ants negatively impact overall seed dispersal in their introduced ranges (Ness 2004). This could either be due to their lack of interest in native seeds, or their typically smaller body size when compared to native ants (McGlynn 1999). Invasive ants can also inhibit important environmental activities indirectly by displacing native ant species (Holway et al. 2002).

Not all ant species become invasive when introduced to a new environment. There are several ecological hurdles that must be overcome for a species to thrive in an introduced range.

One of the most notable obstacles to the successful establishment of an introduced species is known as the biotic resistance hypothesis (Elton 1958). The biotic resistance hypothesis states that introduced species will have a more difficult time establishing when native biodiversity is higher. This is because when the biodiversity is high, most niches and available resources are being occupied or utilized by some existing native species, making it difficult for a new species to establish (Elton 1958). Because of this and other obstacles, a small number of introduced ants ever become invasive. Even then, most invasive species go unnoticed until they develop into a pest.

Despite these obstacles, some ant species exhibit ecological, behavioral, and reproductive characteristics that enable them to quickly spread and thrive in introduced habitats (Holway et al. 2002). One of the most important characteristics of invasive ants that is typically rarely seen in non-invasive ant species is unicoloniality (Holway et al. 2002, Tsutsui and Suarez 2003). Species that exhibit unicoloniality have greatly reduced or absent intraspecific aggression, meaning workers from different nests will cooperate with one another. This allows adjacent nests to freely exchange resources and greatly expand their territory over potentially enormous areas of the introduced range. Another adaptation well suited for invasive success is colony budding. This occurs when queens mate in or near the nest, and form new colonies by moving across the ground instead of flying. Both of these behavioral adaptations together are known as the invasive ant syndrome (Cremer et al. 2008).

An emerging invasive species that has recently attained pest status in the southern United States is the previously mentioned tawny crazy ant *Nylanderia fulva*. The behavior of this ant species appears to encompass both symptoms of the invasive ant syndrome. No *N. fulva* nuptial

flights have been observed, and colonies are composed of nearby nests between which workers freely move (LeBrun 2013).

Initially, this and other crazy ant species were placed in the genus *Paratrechina*. During this time, what is now known as *Nylanderia fulva* was given the name *Paratrechina near pubens*, as it morphometrically and genetically greatly resembled the Caribbean crazy ant *Paratrechina pubens* and other closely related species. However, in 2010 almost all species from *Paratrechina* were placed into the genus *Nylanderia* (LaPolla et al. 2010). During this period of taxonomic uncertainty, *N. fulva* became an increasingly substantial nuisance pest in the southern Gulf Coast area.

Due to *N. fulva*'s recent emergence as a pest, we still lack effective control methods against this aggressively invading ant (Meyers 2008, Gotzek et al. 2012). Ant control typically relies on the use of attractive baits impregnated with a slow acting insecticide. This allows the workers to bring the bait to the colony, where it is shared with the queen and other nest mates via trophallaxis before killing them (Rust and Su 2012). Typically, with proper treatment, this method of treatment can result in complete colony elimination in other ant species. However, even though tawny crazy ants do readily recruit to baits, the colonies still persist after exposure (LeBrun et al. 2013). This is thought to be the result of several factors related to the biology of tawny crazy ants: their high degree of polygyny, meaning they contain multiple queens in a single nest and their polydomous nature, meaning ants move freely between adjacent nests. Although baits can reduce the number of workers and noticeable foraging, tawny crazy ant populations will soon recover as it is unlikely every queen present in the many nests they form will be exposed to the bait (MacGown and Layton 2009). Even if tawny crazy ants are successfully removed from an area, neighboring populations may quickly move in (MacGown

and Layton 2009). Spraying contact and residual pesticides is similarly ineffective. Although these methods can result in high worker mortality, worker populations can quickly resurge to previous levels.

Tawny crazy ants (*Nylanderia fulva*) are members of the subfamily Formicinae. As with the rest of Formicinae, tawny crazy ants do not possess a stinger, and utilize an acidipore or small opening at the tip of their abdomen. When attacking, these ants will spray formic acid from their acidipore (Klotz 2008). In their invasive range, these ants overwinter underground and almost completely disappear in the fall and winter months and do not start to emerge until late spring (personal observation). Unlike red imported fire ants, tawny crazy ant colonies are hard to keep in a lab setting. This impedes research during the fall and winter when these ants are difficult to collect. Part of my research aims to aid research facilities in their ability to keep tawny crazy ants in a lab setting for extended periods of time. This will allow important research to be conducted on this invasive species year-round. The focus of my research was on a suitable diet for rearing colonies of *N. fulva*. For a diet to be suitable, it must contain adequate nutritional content to allow colonies to grow and reproduce. This can be evaluated by measuring worker mortality, reproductive rates such as brood production, and change in weight of the colony.

In their introduced range, tawny crazy ants form polygynous colonies that typically nest in moist soil, including under hard surfaces such as rocks, boards, and flowerpots, in debris, and near tree roots or other structures sunk into the soil that can retain moisture (personal observation, June 2016). Colonies exhibit little to no intraspecific aggression, similar to the Argentine ant *Linepithema humile* (Horn et al. 2013; Suarez et al. 2002). Cooperation between nests allows *N. fulva* to heavily infest areas by reducing worker mortality due to an apparent lack of intraspecific aggression, and increasing foraging productivity (Hölldobler and Wilson 1990).

As a result, *N. fulva* can quickly dominate an area where they establish. This is accomplished primarily by outcompeting neighboring ant species and superior recruitment to resources (LeBrun et al. 2013).

Although it is an ecologically dominant species in its invasive range, the red imported fire ant *Solenopsis invicta* is currently being displaced by *N. fulva*. In addition to the larger colony size and superior recruitment discussed above, *N. fulva* exhibits a remarkable ability to detoxify *S. invicta* venom. Fire ant venom is a potent topical insecticide that workers will attempt to dab or smear on the cuticle of their opponents. However, when *N. fulva* comes into contact with fire ant venom, they curl the tip of their abdomen so that it is touching their mandible then spray formic acid, which they will then use to groom themselves. The spreading of formic acid along the cuticle reacts with the fire ant venom, neutralizing its insecticidal effect (LeBrun 2014). This ability to detoxify venom is incredibly rare, and has most likely evolved in *N. fulva* due to close association and competition between these two species in their native range (LeBrun 2014).

Because *N. fulva* maintains large colony sizes, and recruits workers effectively to resources, they are able to drastically lower ant species diversity within the invasive range (LeBrun 2013). However, even though the number of ant species is reduced, the total biomass of the ant community in the area increases, sometimes by as much as two fold (LeBrun 2013). This indicates that not only is this species able to outcompete other ant species, but they are more effective at utilizing local resources.

Interestingly, *N. fulva* activity does not affect all ant species equally. There seems to be a non-random displacement effect that more heavily targets larger-bodied ant species. In central Texas, the native ant species tend to be larger bodied than the invasive ants. While tawny crazy

ants displace and lower overall ant diversity in their invasive range, the typically larger-bodied native species of ants are eliminated at a higher rate (LeBrun 2013). This leaves a higher proportion of other small bodied, typically invasive species almost untouched (LeBrun 2013). While indirect effects such as competitive advantage and more efficient resource management may be contributing factors to tawny crazy ant's ability to displace other ant species, it may not adequately explain the greater displacement effect on large bodied ants. While we know *N. fulva* are highly aggressive and specialized to combat several *Solenopsis* species (LeBrun 2015), it is unclear how *N. fulva* reacts to other species of ants during physical confrontation. Thus, another major goal of my work was to investigate the levels of aggression exhibited by *N. fulva* toward other ant species that vary in body size. In addition, this study aimed to elucidate possible explanations of differing aggressive displays from *N. fulva*, specifically the size of the ants, their phylogenetic relatedness to *N. fulva*, or their evolutionary or historic interaction with *N. fulva*.

## CHAPTER II

# AGGRESSION OF *NYLANDERIA FULVA* TOWARD COMPETING ANT SPECIES: BIGGER BODIES ELICIT BIGGER FIGHTS

### *Introduction*

The introduction of a new species can have severe ecological impacts on an ecosystem (Lodge 1993). Although species expansions and invasions are natural occurrences, human activity can rapidly accelerate this, forcing species typically separated by thousands of miles to suddenly compete with one another (Hengeveld 1989). Not all species, however, are capable of successfully establishing when introduced to a new environment or range (Lodge 1993). Some taxonomic groups are more successful invaders than others due to various characteristics such as morphology, abundance, and how the invasive species interacts with its environment (Williamson and Fitter 1996). Because ants are social insects, they possess certain characteristics such as cooperative or altruistic behavior and colony size that predispose them to successful invasions of new environments (Moller 1996, Williamson and Fitter 1996).

When invasive species successfully colonize a new environment, their activity has the potential to impact the native biological community in many ways. The impact of invasive species can be measured by analyzing the effect on native communities, such as species richness and diversity, and the changes to native populations (Parker et al. 1999). The effect of invasive species on native species diversity and density are usually explained by indirect ecological effects of invasive activity, such as efficient resource utilization, habitat modification, or population density (Parker et al. 1999). As a result of these indirect effects, native species may be displaced or driven to local extinction by invasive species.

An example of this can be seen in invasive ants such as the Argentine ant, *Linepithema humile* in California (Human and Gordon 1997). Areas where Argentine ants have invaded exhibit drastically decreased native biodiversity with respect to ants and other arthropods (Human and Gordon 1995). The exact mechanism through which native ants and arthropods are displaced by Argentine ants is not fully understood (Holway et al. 2002). It is clear, however, that their ability to displace native ant species stems from their ability to successfully recruit to and defend resources (Human and Gordon 1995), their ability to disrupt local ant foraging (Human and Gordon 1999), and their high population density when compared to native ant species (Rowles and O'Dowd 2007).

The colony structure and behavior of the Argentine ant enables it to produce high population densities. Argentine ants exhibit no intraspecific aggression, likely due to the lack of genetic diversity present in the introduced range (Tsutsui et al. 2000). This allows nearby nests to cooperate as workers can move freely between them, a colony structure known as unicoloniality. This cooperation, along with the polygynous colony structure allows Argentine ants to produce a large number of workers (Tsutsui et al. 2000). Although Argentine ants are initially more aggressive in a one on one fight, they are more likely to be defeated by competing species in an even fight (Buczkowski and Bennett 2008). It is through their high population density and aggressive behaviors towards other species that Argentine ants are able to out compete and displace local species (Buczkowski and Bennet 2008).

Unicoloniality and its implications for invasive success are not unique to Argentine ants. Several ant species display a collection of behaviors known as the 'invasive ant syndrome' (Cremer et al. 2008). Ant species with invasive ant syndrome spread by colony budding, where queens mate underground, and spread on foot instead of taking flight (Holway et al. 2002).



Colonies of ants with invasive ant syndrome also exhibit little to no intraspecific aggression between nearby nests.

A new invasive species to the southeastern United States, tawny crazy ants (*Nylanderia fulva* Mayr) displays the symptoms of invasive ant syndrome, and is impacting the ecosystems of the Gulf Coast. First reported in Florida in the 1950's and detected in Texas in 2002, *N. fulva* has rapidly spread across the Gulf Coast. Much like the Argentine ant, this invasive species is polygynous (LeBrun et al. 2013). Additionally, *N. fulva* does not appear to exhibit intraspecific aggression between nests in the introduced range (Horn et al. 2013). This allows *N. fulva* to outcompete and displace ant species already present in the environment by forming very high population densities within the introduced range (LeBrun et al. 2013). Interestingly, when *N. fulva* invades an area, the ants displaced tend to be larger bodied species (LeBrun et al. 2013). The reason for this higher rate of displacement of larger ant species is unknown, though it is noted that native species tended to be larger than cosmopolitan or other invasive species. While *N. fulva* has been shown to rapidly recruit and defend resources, as well as more effectively utilize resources, it is unclear why these indirect ecological effects would more negatively impact larger bodied ants (LeBrun et al. 2013).

Despite the importance of understanding why some species are successful invaders, the direct interactions between invasive and local species are often overlooked (Buczowski and Bennet 2008, Rowles and O'Dowd 2006). As a result, specific mechanisms by which local species are removed from an area are largely unknown (Rowles and O'Dowd 2006, Tsutsui et al. 2000). In this study, we attempt to determine the mechanisms underlying this heavy targeting of large bodied ants by investigating how *N. fulva* directly interacts when it comes into contact with other ant species. For this investigation we examined interactions between *N. fulva* and ant

species, both native and invasive, found in central Texas. The species we examined comprise a range of body sizes, phylogenetic relatedness to *N. fulva*, and historical association with *N. fulva*. Through this study we attempted to elucidate a mechanism by which *N. fulva* is able to displace established ant species.

### *Materials and Methods*

#### *Collection of Ant Species*

Live tawny crazy ants were dug from nests sourced from Carbide Park in LaMarque Texas. This area is a well-manicured and maintained pecan tree orchard approximately one-half acre in size managed by Galveston County Parks and Senior Services. Collections took place at the base of the pecan trees where the ants had formed their nests among the roots. In order to determine aggression of non-nestmates, tawny crazy ants were also collected from Silver Horse Ranch in Bryan, Texas. The ants were placed in 5 gallon buckets lined with baby powder to prevent escape and transported to the Rollins Urban and Structural Entomology Facility in College Station, Texas. The collected ants were extracted from the bucket by slowly dripping water into the soil to encourage the ants to move upward out of the soil by the rising water level. During this time, the ants were provided harborage by placing five 10 cm x 10 cm corrugated cardboard squares on top of a wire frame. As the water level in the bucket rose and drove the ants out of the soil, they amassed in the cardboard for easy placement into their arenas.

*Solenopsis invicta* workers were collected in College Station, Texas from an open field behind the Rollins Center for Urban and Structural Entomology. *S. invicta* workers were extracted from the soil in a similar manner as the one mentioned above. However, due to their rafting behavior, the ants were skimmed from the top of the water using a slotted metal spatula, without the need

for cardboard harborage. Workers of big headed ants (*Pheidole dentata* and *Pheidole hyatti*), red harvester ants (*Pogonomyrmex barbatus*), cheese ants (*Forelius pruinosus*) and dark rover ants (*Brachymyrmex patagonicus*) were all hand collected from their colonies in College Station, Texas, with the exception of workers of *P. barbatus*, which were collected in Brazos County outside of Kurten, TX. Ants were then placed into plastic shoeboxes lined with Fluon. When needed for study, these ants were aspirated individually from their respective shoeboxes. Workers of arboreal species such as carpenter ants (*Camponotus pennsylvanicus*) and elongate twig ants (*Psuedomyrmex gracilis*) were collected by either aspirating directly from the tree, or by shaking the tree and catching the falling ants in a 5 gallon bucket lined with talcum powder. Ants were maintained at 27 °C under a 14:10 L:D photoperiod and provided with water, 1 M sucrose solution, and an artificial diet (Dussutour et al. 2008).

#### *Aggression Assay*

Arenas were prepared by applying fluon to the sides of 5 cm Petri-dishes. Interactions were recorded by attaching a Canon Vixia G20 video camera mounted directly above the arenas. Arenas were housed in a white poster-board box. Two sides of this box were removed and replaced with diffusion fabric to allow for even lighting, which eliminates shadows on the recording. The recording box and Petri-dish arenas were placed on top of white paper sheets in order to provide contrast for the video recording. Interactions were performed by placing two tawny crazy ants in each arena. A single ant of a different species or nest (the aggressor) was then added to each arena. The interactions were allowed to continue for 1 min after the tawny crazy ants made contact with the aggressor. Aggression was measured on a 0 to 4 scale (0- Stationary, no alarmed response or contact, 1- contact and antennation, 2-contact and immediate retreat, 3- contact and threatening display, such as open mandibles, 4- fighting, biting, spraying

defensive compounds) (Crossland 1990). An aggression score was given to each tawny crazy ant every 10 s for the duration of the 1 min interaction. Only the score of the most aggressive action was taken for each 10 s period. As a control, *N. fulva* aggression was measured against nest mates to determine a base level of aggression.

#### *Body Size and Phylogenetic Measurements*

Ant head capsule width was determined using a wedge micrometer (Porter 1983). Ant heads were removed from the ants and moved down the micrometer. The width was recorded where the ant head could no longer move down the wedge. Phylogenetic relatedness was determined using 18S sequences from GenBank aligned in MEGA7 (Kumar et al. 2016). Phylogenetic distances from *N. fulva* were inferred from pairwise base pair changes.

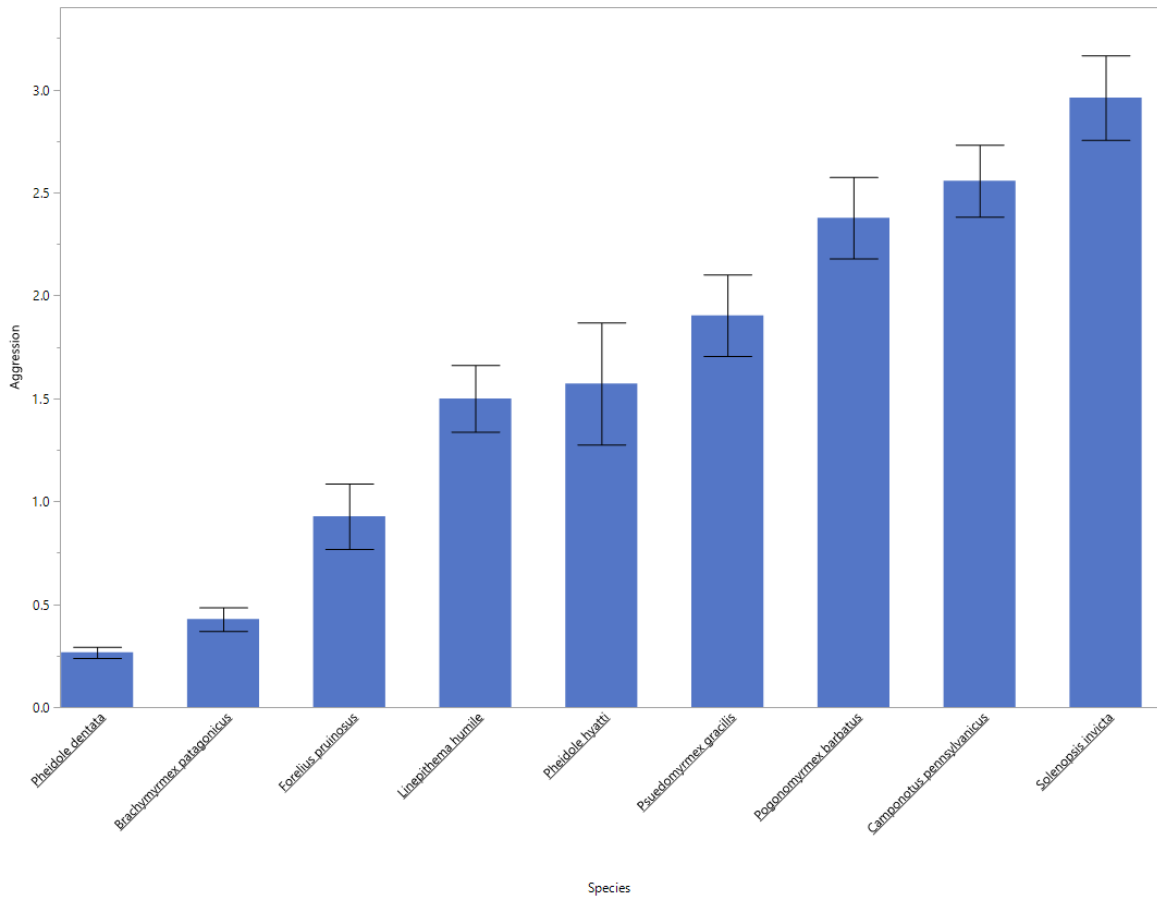
#### *Statistical Analysis*

Aggressor head capsule width, phylogenetic relatedness to *N. fulva*, and historic interaction with *N. fulva* was analyzed using Kruskal-Wallis tests ( $\alpha = 0.05$ ) to determine significant effects on *N. fulva* aggressive displays. If a significant effect of treatment was found, a Tukey's HSD was performed. All possible models were analyzed to determine interactive effects between the three variables. A stepwise analysis was performed to determine R squared and AIC values of all models. All analyses were run in JMP

### *Results*

In aggression tests, *N. fulva* workers quickly displayed differences in their aggression response based on the species they interact with. We hypothesized that *N. fulva* aggression towards other ant species would be determined by phylogenetic relationship, historical

interaction, or size of the other species. Overall, *N. fulva* exhibited the highest aggression when encountering *S. invicta* (Fig. 1).

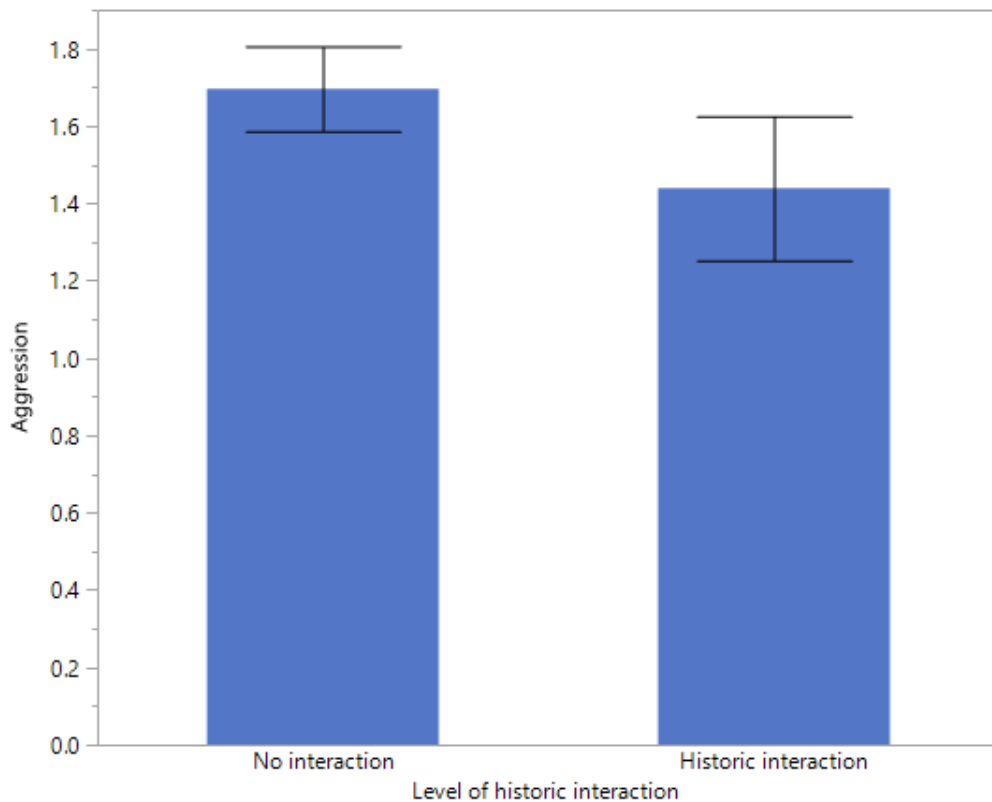


**Fig. 1.** Average of *N. fulva* worker aggression scores. *N. fulva* was most aggressive when encountering *S. invicta* workers, and were least aggressive when encountering *P. denata* workers

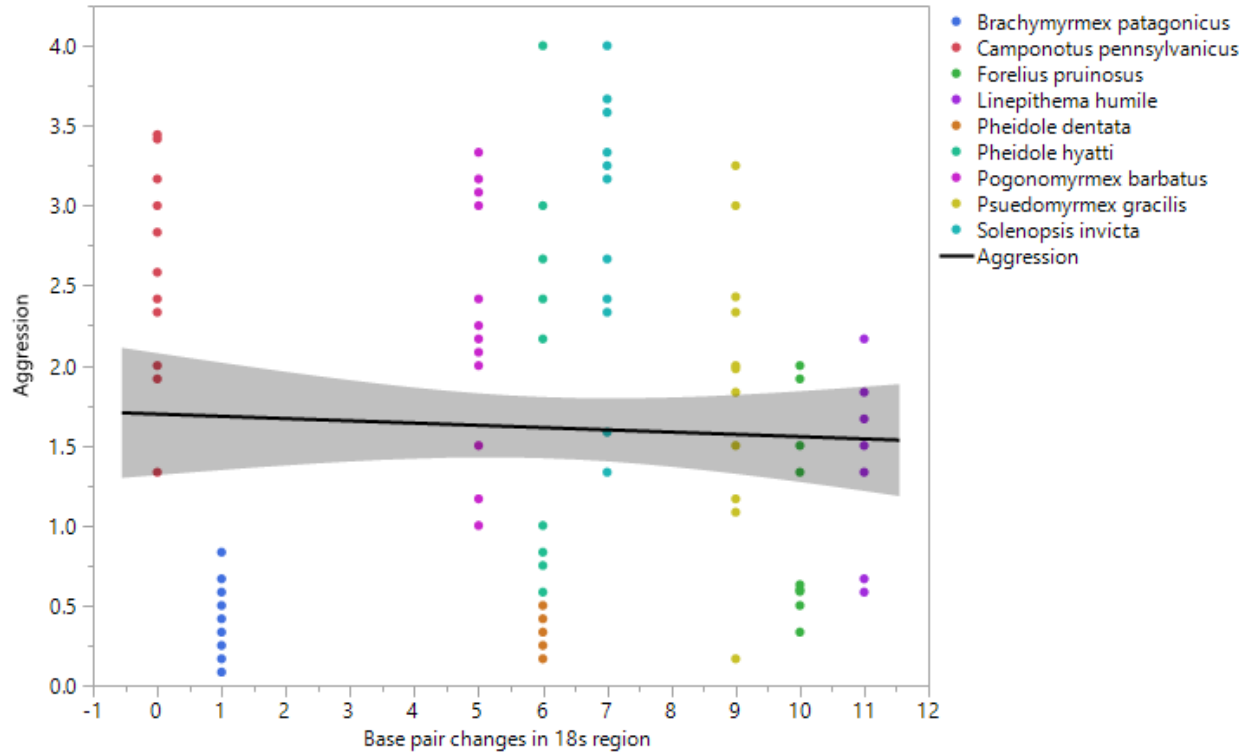
When analyzing the aggression scores with respect to historical interaction (ants that are native to the same area or not) our results show that the levels of *N. fulva* aggression were not significantly different between species that they have coevolved with and species they have been

introduced to in their invasive range (Fig. 2) ( $P = 0.6189$ , Kruskal-Wallis). The species native to the same area as *N. fulva* includes *S. invicta*, *L. humile*, and *B. patagonicus*, and all are relatively small-bodied species. All other species tested are native to North America or Central and North America.

We found no significant correlation of the aggressive response of *N. fulva* based on genetic relatedness. Base pair changes in the 18s region between *N. fulva* and competing species show no change in *N. fulva* aggression ( $P = 0.6070$ , ANOVA) (Fig. 3).



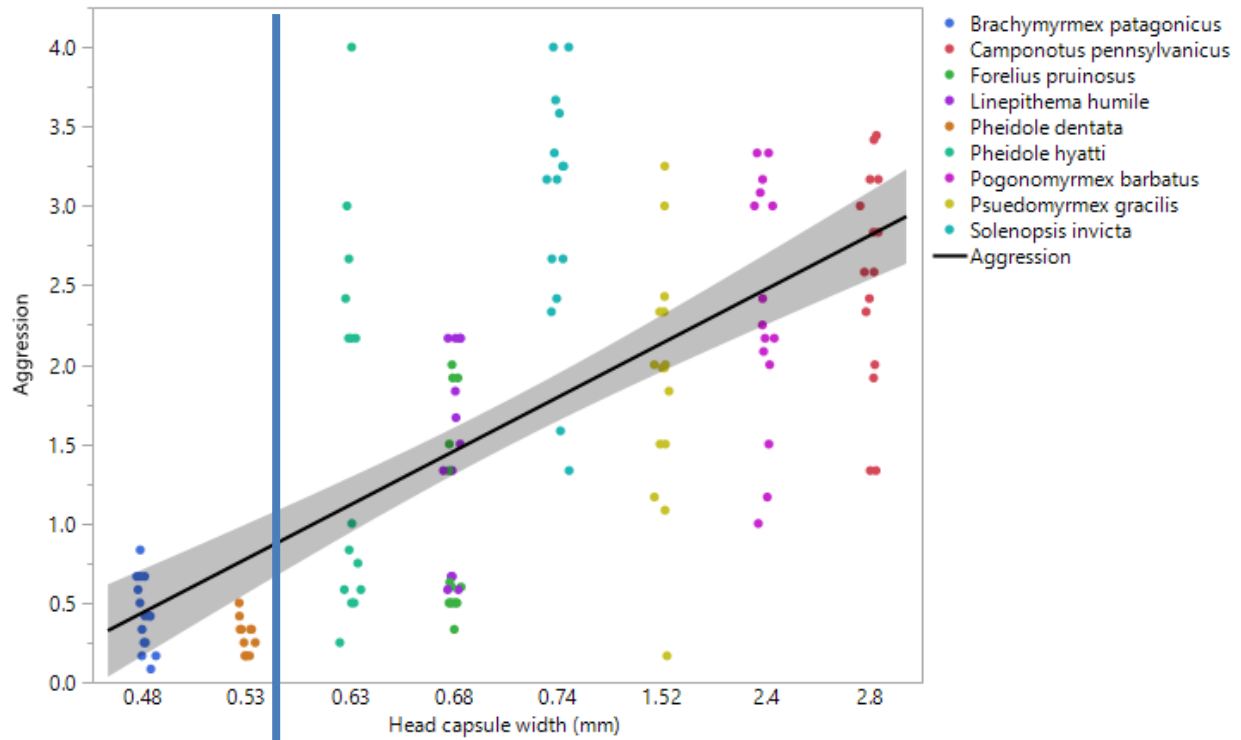
**Fig 2.** *N. fulva* aggression between species it has historically interacted with and species encountered in the invasive range. *S. invicta*, *L. humile*, and *B. patagonicus* coevolved with *N. fulva* while the other species are native to North or Central America. No significant difference was found in the aggressive response of *N. fulva* to the two groups ( $P = 0.6189$ , Kruskal-Wallis test)



**Fig 3.** Aggression display values of *N. fulva* vs phylogenetic distances of species tested. Distances are based on pairwise base pair changes in the 18S region between *N. fulva* and the other species.

*N. fulva* aggression shows the greatest correlation with size of the other species.

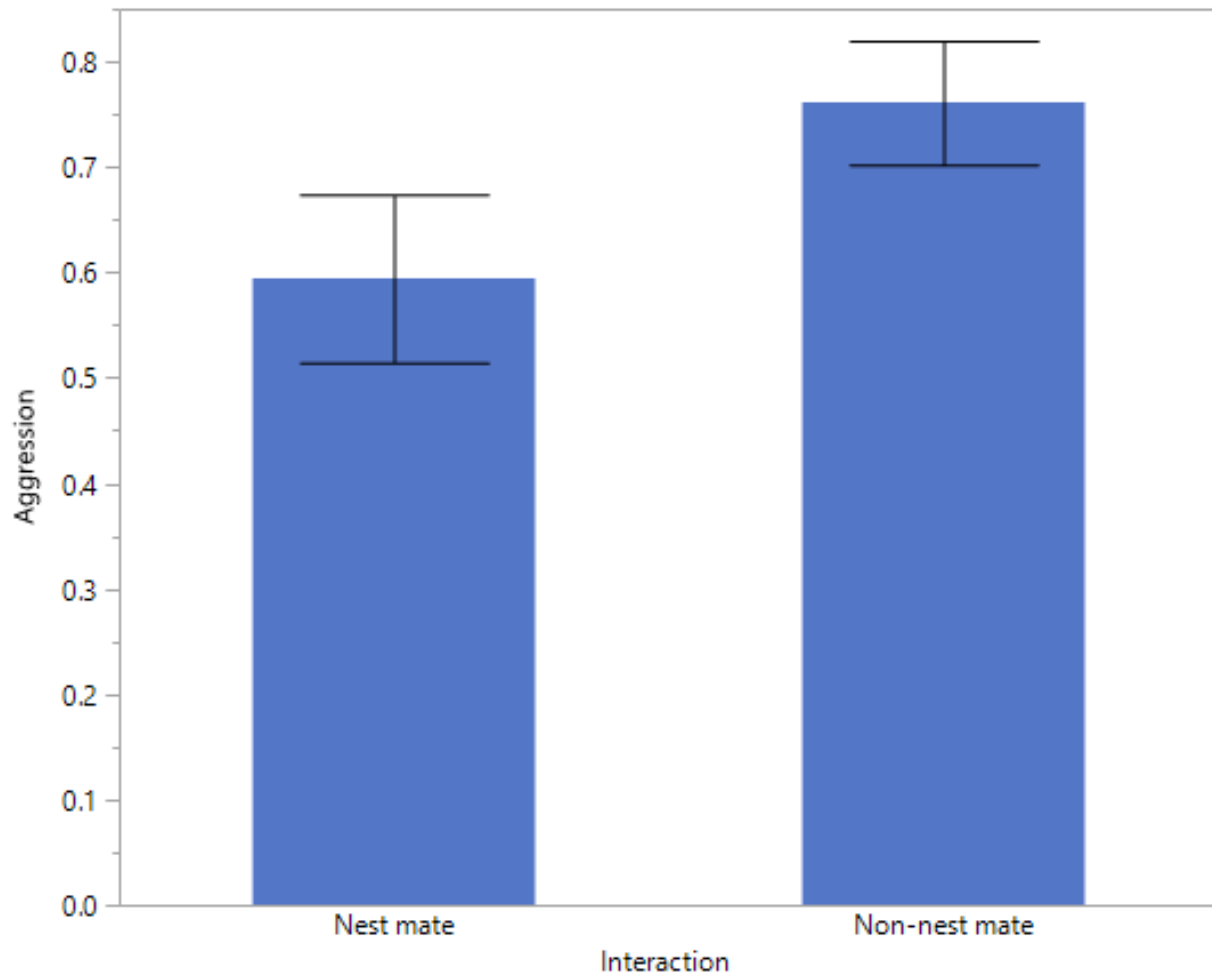
Aggression scores were significantly higher when *N. fulva* workers interacted with larger species (Fig. 4). Large bodied ants were immediately met with aggression and attack by *N. fulva*, while workers seemed to either briefly antennate or ignore smaller species such as *B. patagonicus* or *P. dentata*. The largest species tested was *C. pennsylvanicus* with a head capsule width of 2.8 mm, while the smallest species in this study was *B. patagonicus* with a head capsule width of 0.48 mm.



**Fig. 4.** The average aggression scores of *N. fulva* workers when encountering various species based on head capsule width. The vertical line indicates the head capsule width of *N. fulva* (0.54 mm). Aggressive responses by *N. fulva* workers sharply increased against competing species with larger head capsule sizes.

The intraspecific aggression response of *N. fulva* was analyzed between both nest mates and non-nest mates. No significant differences were found in aggression levels of *N. fulva* sourced from the same colony or differing colonies ( $P = 0.115$ , Kruskal-Wallis test) (Fig. 5). While there was no increased aggression towards non-nestmates, they *N. fulva* workers did spend more time interacting with non-nestmates by antennating them. A lack of significant difference in aggression levels may be due to a low power in the test.





**Fig. 5.** Interactions between *N. fulva* nest mates and non-nest mates. The increased aggression score of non-nest mates is a result of prolonged antennation, and no aggressive actions occurred between interacting conspecifics in any replication.

### *Discussion*

In this study, we attempted to analyze how *Nylanderia fulva* reacts behaviorally when encountering competing ant species present in Central Texas. The ants we chose for our study represent a range of phylogenetically and morphologically diverse species, as well as species both native and invasive to Central Texas. Our results show that *N. fulva* workers react most

aggressively toward *Solenopsis invicta* and *Camponotus pennsylvanicus* workers. Interestingly, our results also demonstrate a marked lack of aggression or even interaction with *Pheidole dentata* or *Brachymyrmex patagonicus*. When analyzing our results, we looked for a correlation of *N. fulva* aggression with several aspects of the species tested: their relatedness to *N. fulva*, their historic interaction with *N. fulva*, and finally their body size.

Our results show *N. fulva* aggression was most weakly correlated with historic interaction, and highly correlated with the size of the ant species *N. fulva* encountered, with *N. fulva* exhibiting high levels of aggression to larger ant species. *N. fulva* displays a remarkable chemical and behavioral ability to combat and displace *S. invicta* colonies in their invasive range (LeBrun et al. 2016). Because of *N. fulva* and *S. invicta* share a native range it is thought that this detoxification behavior was developed through their close association throughout their respective evolutionary histories (LeBrun et al. 2015). However, our results show that while *N. fulva* does display heightened aggression toward *S. invicta*, it does not exhibit aggression towards *B. patagonicus*, another ant species native to South America.

Of the traits examined, size of *N. fulva*'s opponent seems to be the most correlated to aggressive behavior. Again, an example of this can be seen in the aggressive response to *C. pennsylvanicus* and *B. patagonicus*. Although these two ants belong to the same subfamily as *N. fulva*, the difference *N. fulva* displayed in aggression was remarkable. *N. fulva* attacked *C. pennsylvanicus* on sight, while only briefly interacting with or completely ignoring the presence of *B. patagonicus*. Perhaps the best example of the disparity in *N. fulva* aggression due to size is the aggression displayed towards *Pheidole hyatti* and *Pheidole dentata*. These species belong to the same genus; however *P. hyatti* workers, which produced a higher aggressive response from *N. fulva*, have a head width of 0.63 mm, and are larger than their *P. dentata* counterparts who

have a head width of 0.53 mm. Additionally, *N. fulva* nests have been known to house complete colonies of both *P. dentata* and *B. patagonicus*, including workers, brood, and reproductives (personal observations).

Ant aggression as a function of the opponents' size or the size difference between competing species has not been heavily studied. It has been suggested that some ant species will alter their aggressive behaviors when faced with opponents of differing sizes (Nowbahari et al. 1999, Horn et al. 2013). For example, Nowbahari et al. (1999) showed that *Cataglyphis niger* workers exhibited higher aggression towards smaller bodied ants. Interestingly, our results demonstrate the opposite; *N. fulva* workers are more aggressive towards larger ants. Support for this can be found in the observations of LeBrun et al. (2013), which show that *N. fulva* invasion dramatically reduces the abundance of larger ant species in the invaded area. Although the impact *N. fulva* invasion has on larger-bodied ant species has not been fully explained, our results provide a possible mechanism through the high aggressive behavior displayed by *N. fulva* when directly interacting with these species.

It is curious that the physical size of competing ant species would correlate strongly with aggression, as ants and other social insects are generally thought to strongly rely on chemical communication and cues (Wyatt 2003). Ants utilize cuticular hydrocarbons to determine whether individuals are nest mates or non-nest mates (Singer 1998). Cuticular hydrocarbons are also believed to be closely regulated by the ant's genotype (Lockey 1991, Beye et al. 1998). Therefore, it is surprising that ants more distantly related, such as *P. dentata* would elicit almost no response from *N. fulva* as opposed to their own nest mates, who were at least antennated frequently. One potential explanation for this may lie in the visual cues the ant receives when interacting with another species. It has been shown that ants cannot determine distance of an

object (Via 1997). The bulldog ant *Myrmecia gulosa* respond aggressively to an object when a large amount of their field of vision is obscured, regardless of how far away the object is from the individual (Via 1997). In *N. fulva*, aggression may be linked to both vision and chemical signals detected from other ant species. For example, if an *N. fulva* worker chemically detects another ant, and a large portion of its field of vision is obscured by the ant, aggressive behavior may be triggered.

Our results show no difference in *N. fulva* aggression between nest mates and non-nest mates. The lack of intraspecific aggression in *N. fulva* is typical of successful invasive species in their invasive range. One example is the invasive Argentine ant *L. humile*. This species has formed massive super colonies in California (Heller et al. 2008). Another example of this can be seen in the invasion of *S. invicta* in the U.S. Monogyne colonies contain a single queen, and workers exhibit intraspecific aggression (Holway et al. 2002). However, *S. invicta* colonies in the invasive range tend to produce polygyne or multiple queen colonies with low or absent intraspecific aggression (Tschinkel 1998). The lack of intraspecific aggression is thought to contribute to increased colony size and population density, as it eliminates competition between conspecifics and promotes cooperation and the sharing of resources between nests (Holldobler & Wilson 1990). It is currently unclear if *N. fulva* displays unicolonial behavior in their native range, however the lack of aggression between *N. fulva* non nest mates in their invasive range is also observed in other lab trials and in field studies (Horn 2009).

The ability of *N. fulva* to displace large-bodied ant species is of particular ecological concern to their invasive range in Central Texas. Large ants present in the environment tend to be native, while the smaller ants present are more often invasive (LeBrun et al. 2013). Thus, the displacement effect of *N. fulva* on local ant populations seems to affect native species more than

invasive species (LeBrun et al. 2013). Due to this displacement of native species by *N. fulva*, other small bodied, often invasive species are allowed to increase in density as well (LeBrun 2013). This displacement effect by *N. fulva* not only increases invasive ant abundance, but also leaves the ecosystem more prone to invasion by other ant species due to the ecological disruption and lack of diversity (Hoffmann et al. 1999). The long term impacts of *N. fulva* activity in their invasive range are still unclear (LeBrun et al. 2013).

The aim of this study is to shed light on how *N. fulva* directly interacts with ants present in their invasive range. My results demonstrate variable aggression responses of *N. fulva* when facing local populations of ants across several subfamilies, and provide a possible explanation for their displacement effect on large-bodied species. However, more research should be conducted into the modality of *N. fulva* aggression by interrupting their recognition senses, such as painting over their eyes or clipping antennae, to elucidate the underlying cause of their aggressive behavior towards large ants. Another aspect that warrants further investigation is examining differences in the cuticular hydrocarbon signature between ant species. Because larger ant species have a higher surface area, it may be that they are producing specific hydrocarbons to aid in desiccation resistance. Because recognition is highly influenced by an ant's hydrocarbon signature, differences in large bodied species may induce aggressive behavior in *N. fulva* (Lahav et al. 1999). These results will hopefully provide useful information to areas at risk of *N. fulva* invasion by allowing predictions of which ant species may be reduced or eliminated from the area.

## CHAPTER III

### AN INVESTIGATION OF DIETS TO SUSTAIN COLONIES OF *NYLANDERIA FULVA* IN THE LABORATORY

#### *Introduction*

All animals must consume food as both a source of energy to fuel metabolic processes, and to meet physiological needs. Adequate intake of nutrients is required to effectively perform basic life functions such as growth, maintenance, and reproduction. However, not all food sources are created equally, and animals must actively regulate their food intake to balance optimal levels of nutrients to maintain homeostasis and maximize the effectiveness at which they carry out their various physiological processes (Solon-Biet et al. 2005). In order to reach these optimal nutrient levels, animals can regulate nutrient intake in two ways. When only a single food item is available, animals regulate nutrient levels by the amount of the single food item consumed (Simpson and Raubenheimer 1993). Although this food source may contain all of the essential nutrients required by the organism, the concentrations of these nutrients may not be consistent with the organism's optimal requirements. When this scenario occurs, the animal must consume enough food to meet all limiting requirements, which can lead to an excess consumption of other nutrients (Cook et al. 2010). Depending on the nutrient, such as carbohydrates or protein, negative physiological consequences may arise when consumed in excess. For example, when fed only a single diet with a high protein to carbohydrate ratio, *Drosophila* produce more eggs but experience a shorter total lifespan (Lee et al. 2008). Conversely, when fed a single diet with a high concentration of carbohydrates, and low concentration of protein, the flies laid fewer eggs, but experienced a longer lifespan (Lee et al.

2008). When more than one food item is available, animals can regulate by selecting from various food sources in order to reach physiological optimal nutrient levels (Simpson and Raubenheimer 1993). This allows organisms to meet minimum nutrient levels, while preventing overconsumption of other nutrients and the resulting deleterious effects.

When an animal can choose between different food sources, it may reach optimal nutrient levels, even if the food choices themselves are not optimal. This is accomplished by regulating how much of each food source is consumed (Simpson and Raubenheimer 1993). This is a fairly straightforward behavior when considering a single animal; it may choose to eat more of one food source and less of another to meet minimum nutrient requirements (Simpson and Raubenheimer 1993). The same principles of nutrition, and the effects of imbalanced nutrition, apply for eusocial animals such as ants. When thinking about nutritional needs of social insects, it helps to consider them not as a collection of individuals, but as a single “emergent” entity or “super organism” (Wheeler 1911, 1923). Just as with individual organisms, an imbalanced source of nutrition, such as an excess of protein or a carbohydrate deficiency, can have physiological consequences on the entire colony. These consequences can range from an increase in worker mortality, to reducing the rate of egg-laying and brood production in reproductive castes (Cook et al. 2010). It has been shown that when given a choice of several diets with imbalanced protein to carbohydrate ratios, ant colonies will self-regulate their foraging intensity on the available diets to meet nutritional demands; much like an individual animal would (Cook et al. 2010).

However, because ant colonies are made up of various physiologically distinct castes, not all members of the colony possess the same nutritional requirements. Compounding the situation even more, colonies typically contain overlapping generations, which may also have different

dietary needs (Cook et al. 2010, 2012). In regards to ants, the workers require carbohydrate rich diets as fuel to complete their various activities in and around the colony, such as foraging, brood care, and structural maintenance, while the immatures and reproductive members of a colony require a more protein and nitrogen rich diet for development (Dussutour and Simpson 2009, Arganda et al. 2016). Because different members of the colony have different nutritional needs, and foraging workers are the only members actively searching for and retrieving food, some decision making must take place in order to make sure the colony is not deficient (Holldobler and Wilson 1990). Current research points to several factors, both on an individual and colony level, that influence ant foraging preferences. An individual forager's decisions can be influenced by its current nutritional state, such as its current lipid stores (Blanchard et al. 2000), or the scarcity of the resource in the environment (Kay 2004). Colony level influence of foraging preferences most likely stems from the nutritional needs of developing brood, which is communicated first to the workers nursing them, and then throughout the colony (Behmer 2009). It has been shown that just the presence of brood or larvae in a colony can greatly influence both the colony's rate of foraging, and the diet preferences of foragers (Dussutour et al. 2006).

When rearing insects, it is important to understand why they choose to forage on particular foods, and how the concentration of micro and macronutrients affects their physiology and growth. Insect rearing can be defined as insects "...from colonies or cultures deliberately kept by people rather than collected from fields or from households or stored-product facilities" (Cohen 2001). When insects are reared, typically they are given a single food source. However, a single source of food is rarely representative of a 'perfect food' which means it does not contain nutrients in the correct ratio for optimal insect or colony growth (Cook et al. 2012). Forcing colonies to forage on a single, most likely imbalanced diet may lead to poor colony health. For



example, diets too rich in protein can induce toxicity from high amino acid concentration. In ants, this results in leaner workers with lower lipid reserves, increasing worker mortality (Arganda et al. 2017). Other deleterious colony effects can occur as well. When Williams et al. (1987) attempted to rear *Solenopsis invicta* colonies on a ground beef based diet, they discovered nutrient deficiencies in their artificial diet resulted in the production of non-melanized workers. Another example occurred when Wheeler (1910) fed colonies of *Formica schaufussi* imbalanced diets. These colonies failed to develop mature reproductives when vitamin mixtures were removed from the artificial diet.

Cohen (2001) determined 60% of arbitrarily chosen studies from several entomological journals utilized reared insects. This indicates that the use of reared insects is prevalent in current entomological research. Insect rearing aids researchers by allowing for a constant, large supply of insects for study, making it possible to run multiple concurrent studies on the same species, or making it easy to duplicate studies. Rearing insects can in many cases be cheaper than field collection, reducing or eliminating the man-hours that would be spent in the field to collect an adequate number of insects for study. Cohen goes on to argue that in many papers, insect rearing techniques or diet are not always clearly listed in the methods sections, giving rise to the possibility that the insect subjects may be malnourished, or improperly treated in such a way to alter the results of the study. This can especially affect studies on insect behavior, physiology, or toxicology (Cohen 2001).

Insect rearing is also important when considering studies on insects that cannot be collected year-round, as is the case with my species of interest, tawny crazy ants (*Nylanderia fulva*). Although present in large numbers between late spring and autumn, these ants are very difficult to collect in the cooler parts of the year in their introduced range (personal observation).

This severely limits the time available to conduct research on this species. Although it is an important aspect of entomological study, insect rearing has a “lack of true [scientific] protocols” in the literature, which can impede study on ecologically and economically relevant insect species (Cohen 2001).

Difficulties rearing *N. fulva* have been noted in other entomological research laboratories (Oi, personal communication). After reaching out to Dr. Oi it became clear that an artificial diet, although appropriate for rearing some ant species, may not be sufficient for *N. fulva*. He noted his observations of greater *N. fulva* colony health in the lab when the colonies are fed a live insect diet, specifically termites. However insects can contain differing levels of nutrients (Finke 2012) and may or may not be equally suitable as a diet for captive *N. fulva* colonies. Thus, this research is aimed at determining the most appropriate insect for use as feed to sustain *N. fulva* colonies over the long term, allowing research to be conducted year round on this ecologically relevant invasive species.

## *Materials and Methods*

### *Study Site and Collections*

Groups of *N. fulva* were collected from Carbide Park in LaMarque Texas. This area is a well-manicured and maintained pecan tree orchard approximately one-half acre in size managed by Galveston County Parks and Senior Services. Collections took place at the base of pecan trees where the ants had aggregated among the roots.

Ants were dug from the ground using a shovel and 5 gallon paint buckets lined with baby powder to prevent escape. The ants were then transported to the Rollins Center for Urban and Structural Entomology at Texas A&M in College Station, Texas. The ants were extracted from

the bucket by placing the bucket in a sink and slowly dripping water into the soil, encouraging the ants to move upward out of the soil. During this time, the ants were provided a harborage consisting of five 4 in x 4 in corrugated cardboard squares on top of a wire frame. As the water level in the bucket rose and drove the ants up out of the soil, the ants amassed in the cardboard allowing for easy placement into the containers in which they were housed.

#### *Care and Maintenance of Ants Prior to Experiment*

Once in the lab and extracted from the soil, the cardboard and ants were placed in a 29 cm x 16 cm plastic container lined with fluon (Bioquip). This container was provisioned with a 15 cm test tube filled with water and plugged at the end with a cotton ball. The ants were kept at a 14:16 light:dark cycle and 27 °C for the duration of the study. No food was given to the ants during the 24 h extraction and set up period.

#### *Arena Construction*

Each replication was constructed in a 150 mm Petri dish, lined with fluon. These arenas were provisioned with water and a 1 M sucrose solution which were provided in separate 7.5 cm test tubes and plugged with half a cotton ball to prevent leakage. Water and sucrose were provided *ad libitum*. Two additional small test tubes were filled with 5 mL of water, plugged with cotton, and topped with approximately 5 cm of Castone dental stone (Dentsply). These Castone tubes served as harborage for the ants. The Castone allowed for ample humidity for the ants to use as a nesting chamber, while deterring fungal growth. In addition, a 90 mm Petri dish was placed inverted over the harborage tubes. This was done to help maintain the humidity level in the harborage tubes. A 3-cm section was removed from the outer edge of the small Petri dishes to allow the ants access between the harborage tubes and the rest of the arena. Each replicate, or “colonoid,” was provisioned with 0.5 g of workers (~800 ants) and five queens and their

assigned diets. All ants were sourced from the collection site at Carbide Park in LaMarque, TX over the course of this study.

### *Diet Preparation*

Diets consisted of whole insects, an artificial diet used in the lab for other insect species (Dussutour et al. 2008), no diet, where colonoids were provided only water and sucrose solution, or a combination of artificial and insect diets. The artificial diet was prepared per instructions and stored in Petri dishes at 4 °C (Dussutour et al. 2008). The insect diets were macerated in a mortar and pestle to ensure homogenization, and stored at -20 °C in a sealed plastic container. The insects used as diets were chosen because they are used commonly as diets for various arthropod and non-arthropod species. The species used in this study include crickets (*Acheta domestica*), mealworms (*Tenebrio molitor*), termites (*Coptotermes formosanus*), beet armyworms (*Spodoptera exigua*), and the Canton-S strain of vinegar flies (*Drosophila melanogaster*). Ants were fed adults of termites, crickets, and *Drosophila* and immatures of mealworms, beet armyworms. *Coptotermes formosanus* and *D. melanogaster* were identified by hand, while the other species were sourced from professional rearing facilities. *C. formosanus* workers were sourced from colonies collected in Galveston, Texas. *D. melanogaster* individuals were sourced from Texas A&M research stock colonies.

### *Data Collection*

Each colonoid was fed 0.25 g of diet every 48 h for 6 wk for a total of 21 feedings. Feedings consisted of placing the assigned diet in small labeled weigh boats to allow for easy removal and replacement in the container. The diet removed was weighed to obtain the weight lost due to ant activity, and corrected for water loss. Water loss was determined by placing 0.25 g of diet outside of the container for 48 h. These portions of the diets were then weighed to

determine the water loss that occurred during the feeding periods. Along with the diets, the number of dead workers were also tallied and removed from the colonoids during these feedings. At the end of 6 wk, the final weight of the workers was obtained, as was the weight of any brood produced during the trial, and the numbers of dead workers from each colonoid were totaled.

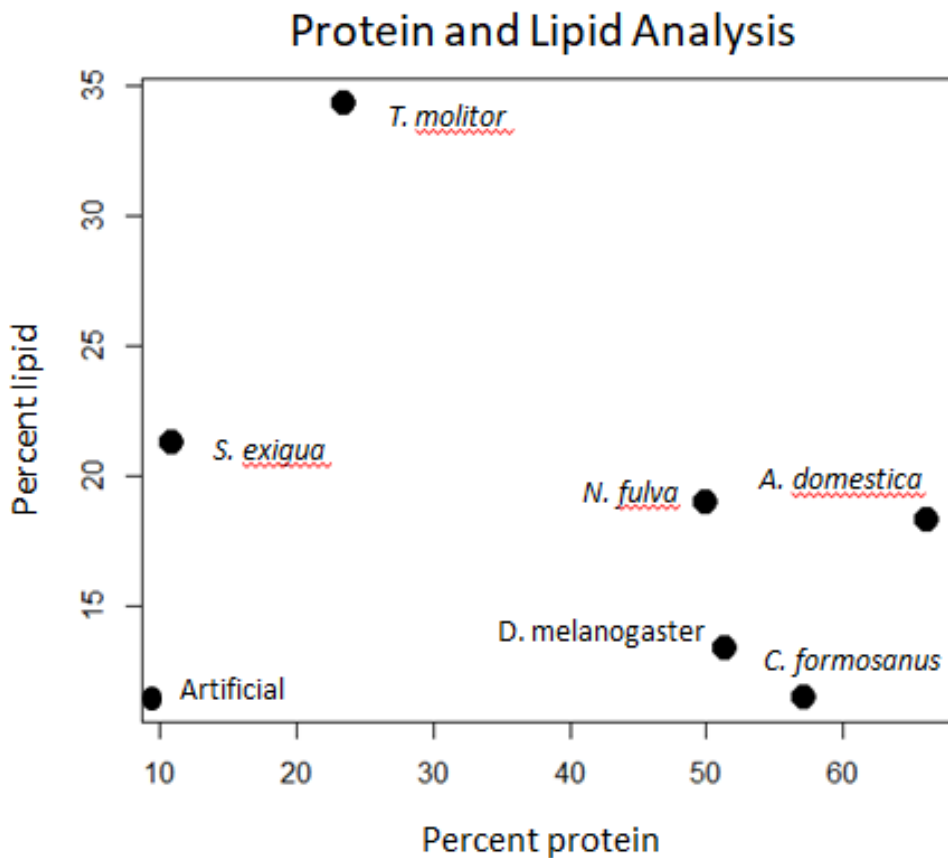
### *Statistical Analysis*

Weight of diet consumed, change in colony weight, total dead ants removed, and weight of brood produced were analyzed using an ANOVA to determine if there were significant differences ( $p < 0.05$ ) of these phenotypes exhibited by the ants due to diet. If a significant effect of treatment was found, a Tukey's HSD was performed. Analysis of the lipid and protein concentration of the diets and ants was performed. Protein concentration was determined by calculating total nitrogen content via high temperature combustion process (Sweeny 1989). Lipid content was calculated by taking the dry weight of the diet before and after two chloroform washes (Simpson et al. 2002). Water content was calculated based on subtraction of dry weight from wet weight. Elemental analyses were also performed on diets and ants following inductively coupled plasma (ICP) spectroscopy protocols (Isaac and Johnson 1975, Havlin and Soltanpour 1989). The protein and lipid concentrations were regressed against each other to create a geometric nutritional framework of the diets tested. This framework was utilized to create splines showing predicted performance of ant colonies (weight loss, brood production, and survivorship) based on ant colony measures of health when fed a diet consisting of a range of protein and lipid concentrations. The number of dead ants removed at each feeding was plotted in a time series to determine differences in diet performance over the course of the experiment.

All statistics were run using JMP, and splines were created using the R fields package (Nychka et al. 2015).

### Results

Nutritional analyses indicate differences in the quality of diets chosen (Table A1). Termites possessed the highest protein to lipid ratio, while beet armyworms had the lowest (Fig. 6). 2a illustrates the total mass of food consumed by the colonies every 48 hours between feedings.

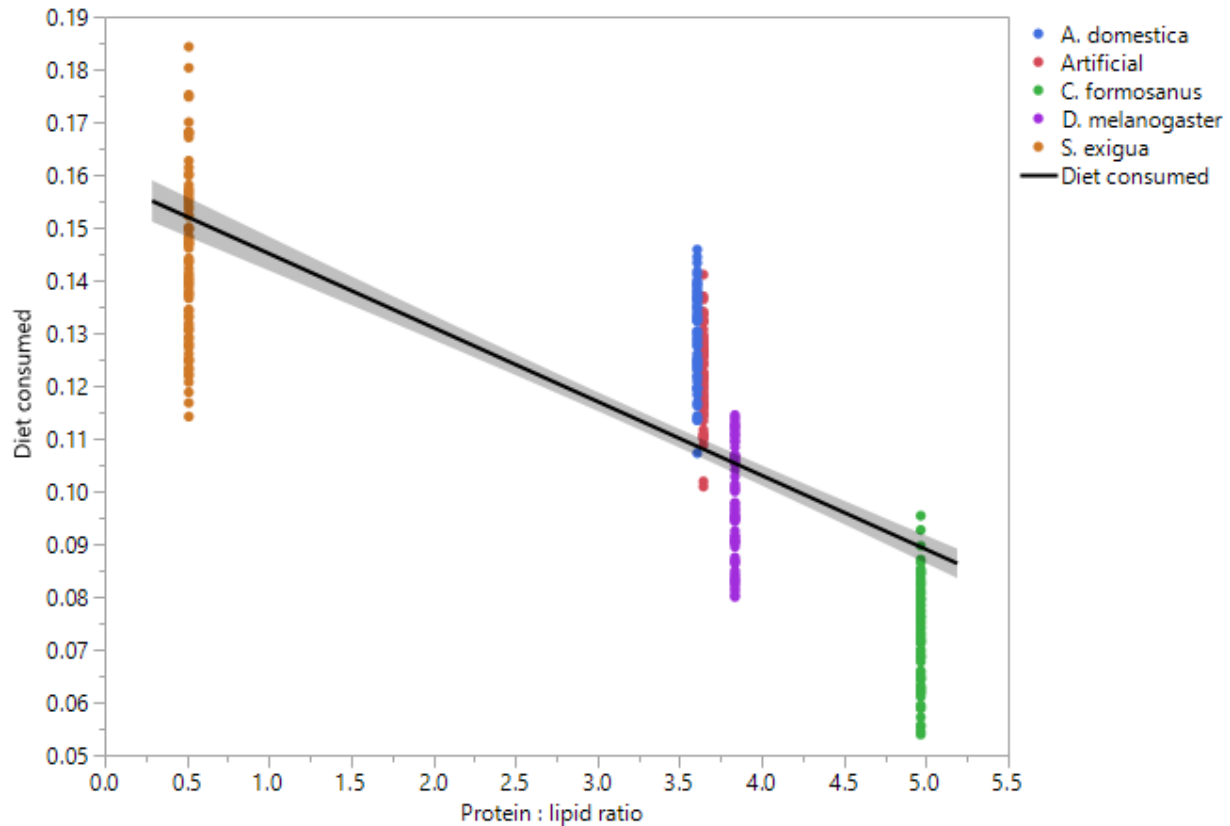


**Fig 6.** Protein to lipid ratio of all diets and *N. fulva*.

By dry mass, beet armyworm experienced the highest rate of consumption, while mealworms experienced the lowest. With the exception of mealworms, increasing the protein :

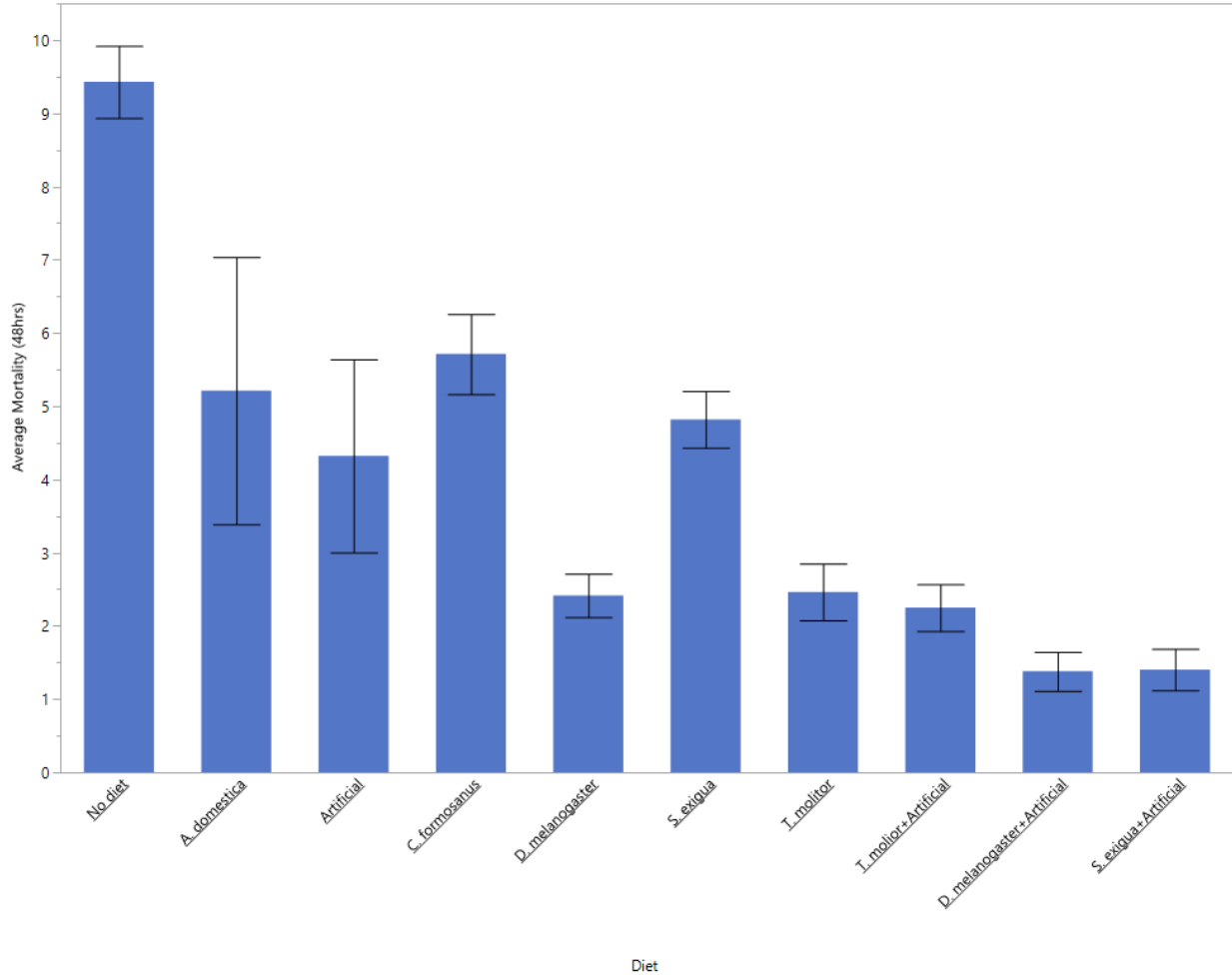
lipid ratio in a diet seemed to cause a decline in the total diet consumed by the ants (Fig. 7).

Protein and lipid content analysis of *N. fulva* revealed a high protein : lipid ratio.



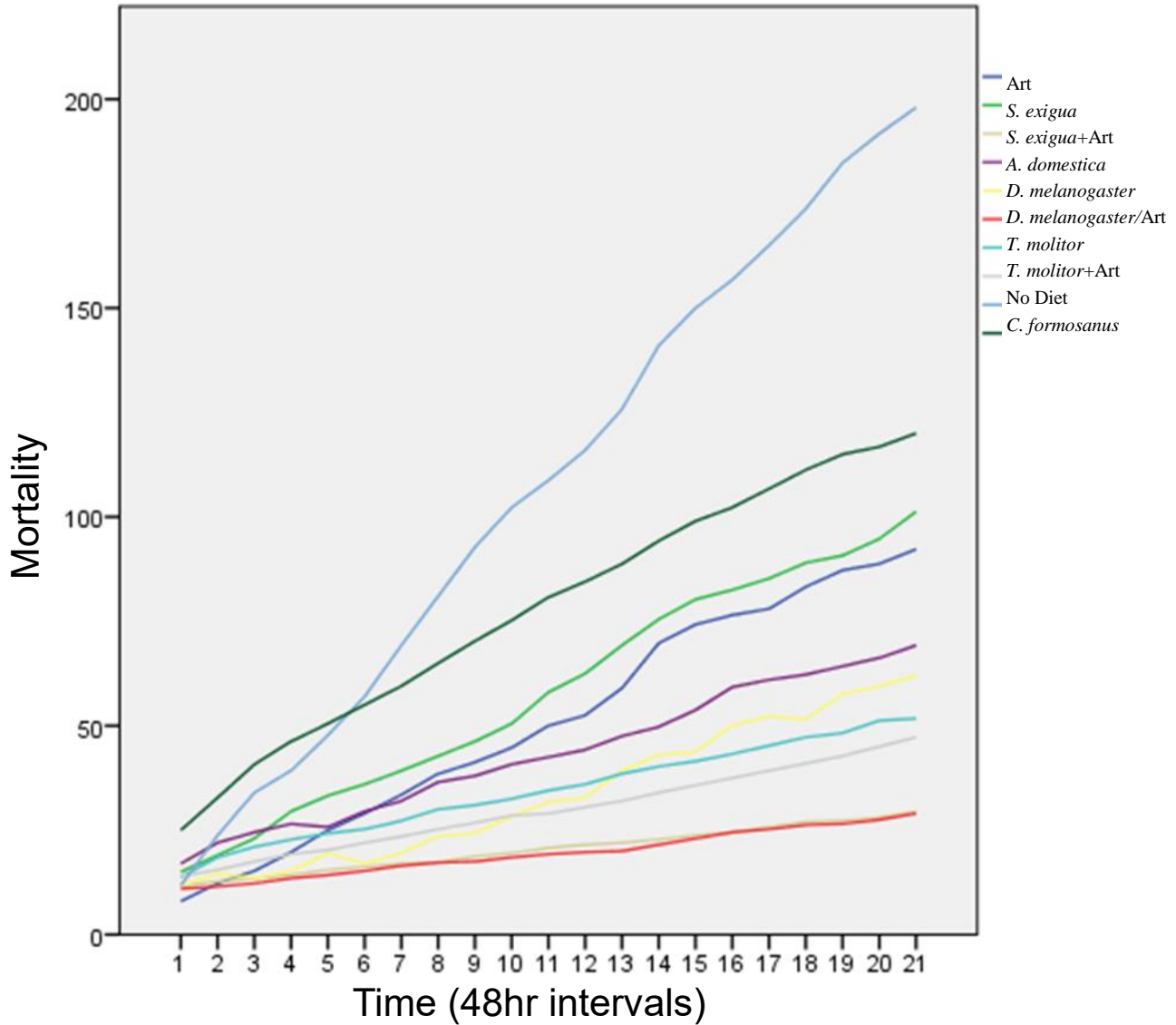
**Fig. 7.** The amount of diet consumed plotted against the ratio of protein to lipids present in the diet. *T. molitor* consumption data was removed from the graph to better illustrate the relationship due to the low rate of consumption of *T. molitor* by *N. fulva*.

The mortality experienced by colonoids that were supplied no diet was significantly lower than all other treatments and experienced the highest average mortality (Tukey's HSD,  $p = 0.001$ ), while mealworm and *Drosophila* experienced the lowest average rate of mortality (Fig. 8). Colonoids denied any diet also experienced the highest rate of mortality over the course of the study (Fig 9).



**Fig. 8.** The effect of diet on the average worker mortality ( $\pm$ SEM) experienced by colonoids. Mortality was recorded at each feeding every 48 hours. Once recorded, the dead workers were removed from the colonoid.

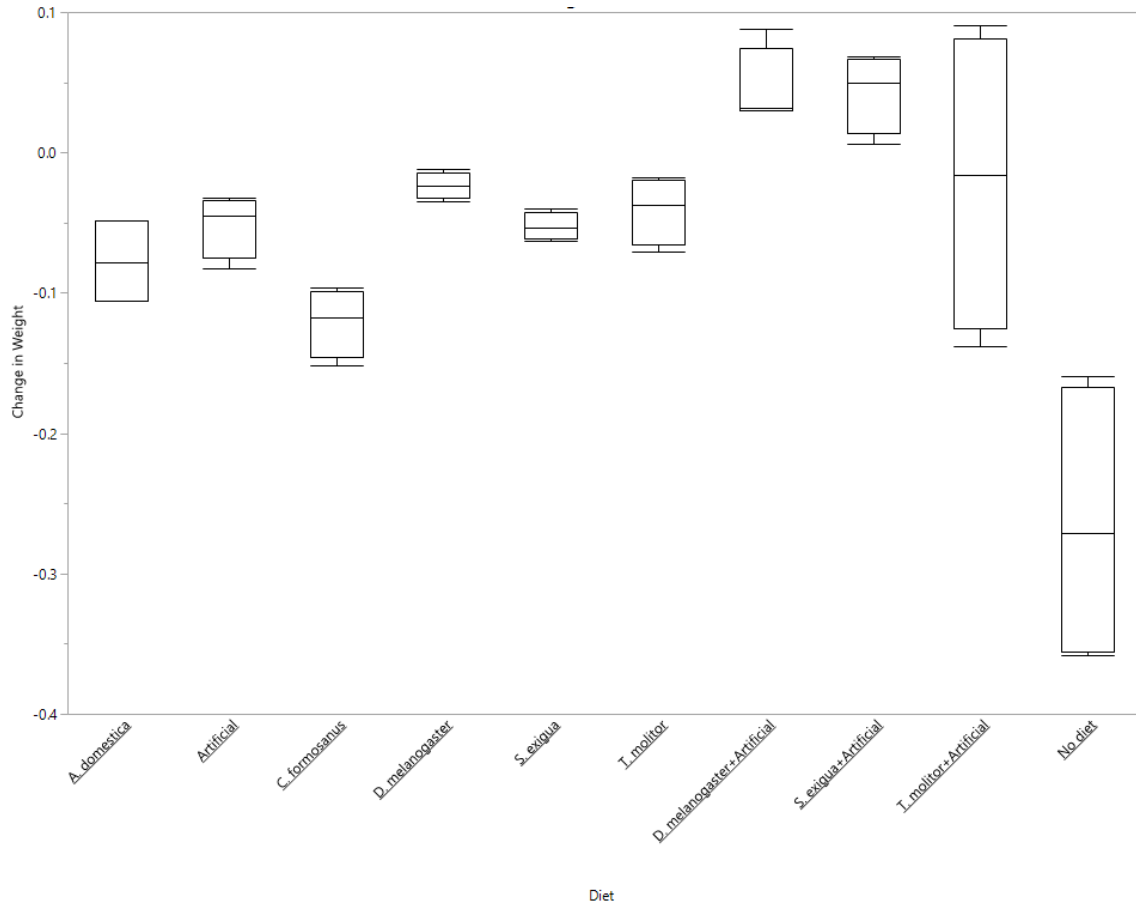




**Fig. 9.** Rate of mortality experienced due to diet. Observation periods occurred at feedings every 48 hours. Dead individuals were tallied and removed from colonoids each observation period. Cumulative mortality is reported. “Art” denotes the artificial diet.

Total colonoid weight was recorded at the beginning and end of the experiment. Colonies fed no diet lost significantly more mass than colonies that were fed any diet (Tukey’s HSD,  $p = 0.0126$ ). Although no diet performed significantly better or worse than other diets in terms of

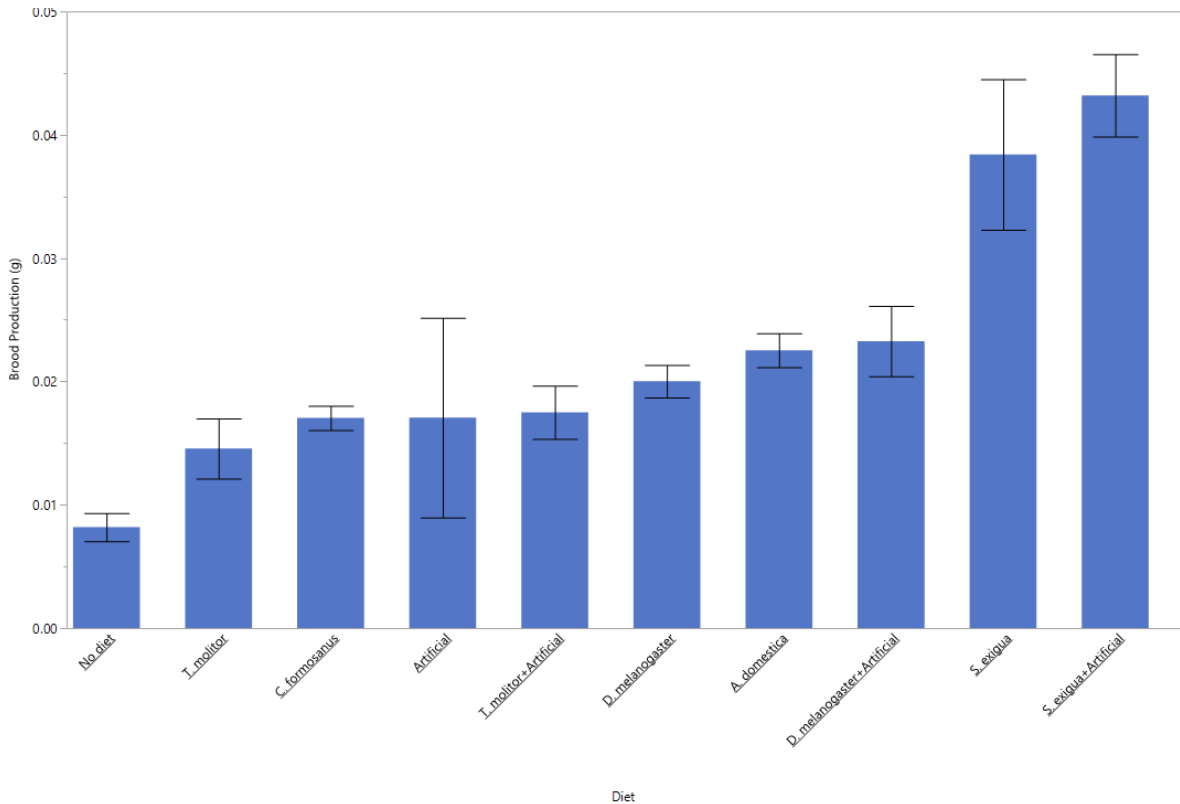
colonoid weight change, colonoids fed *Drosophila* and mealworm on average lost the least amount of weight while colonoids fed termites experienced the greatest weight loss (Fig 10). Calcium, copper, and Manganese content in the diets correlated negatively with weight (Fig. A-6).



**Fig. 10.** Effect of diet on the change in weights of colonoids during the course of the experiments.

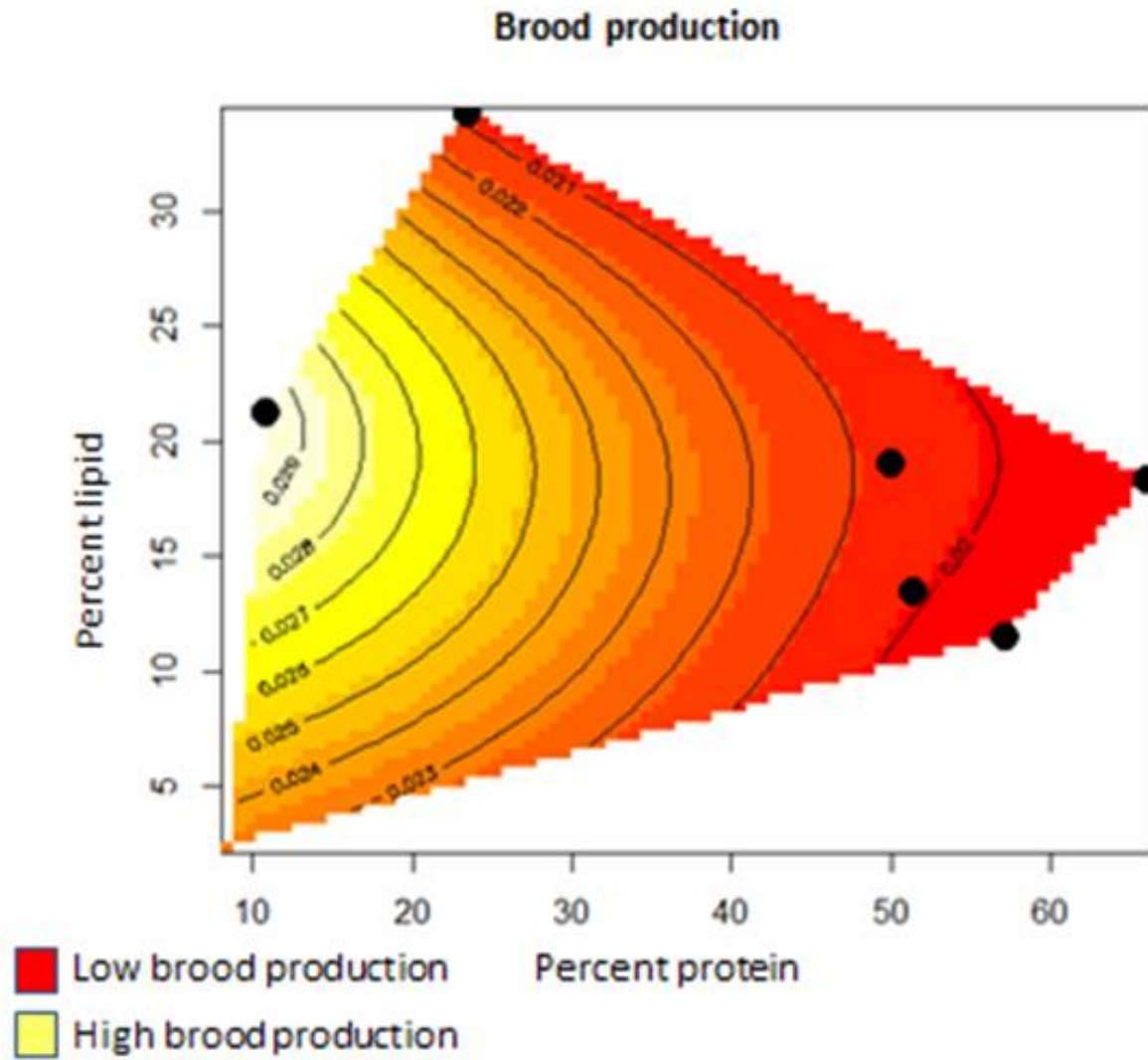
At the conclusion of the experiment, colonies which were fed beet armyworm produced significantly more brood than colonies fed any other diet (Tukey's HSD,  $p = 0.0001$ ) (Fig. 11). Although not significantly different from any other diet, colonies fed with crickets produced the

least brood on average ( $\alpha = 0.05$ ). Colonies fed no diet produced significantly less brood than colonies that were fed any diet (Tukey's HSD,  $p = 0.002$ ). Micronutrients phosphorous, calcium, sulfur, and boron all correlate negatively with brood production (Fig. A-7).

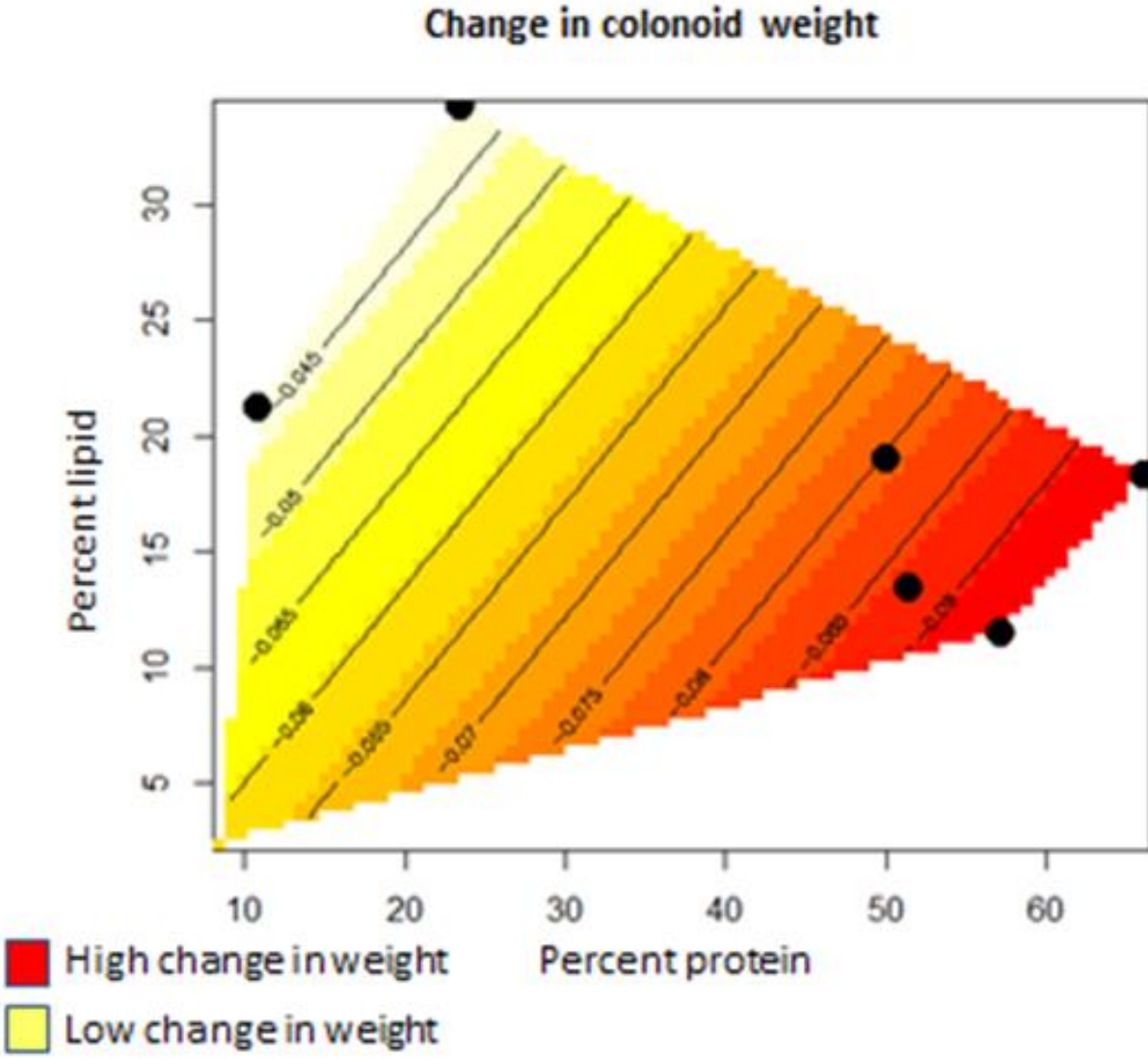


**Fig. 11.** Brood production at the conclusion of the study. All developmental stages of brood were removed and weighed to determine brood production

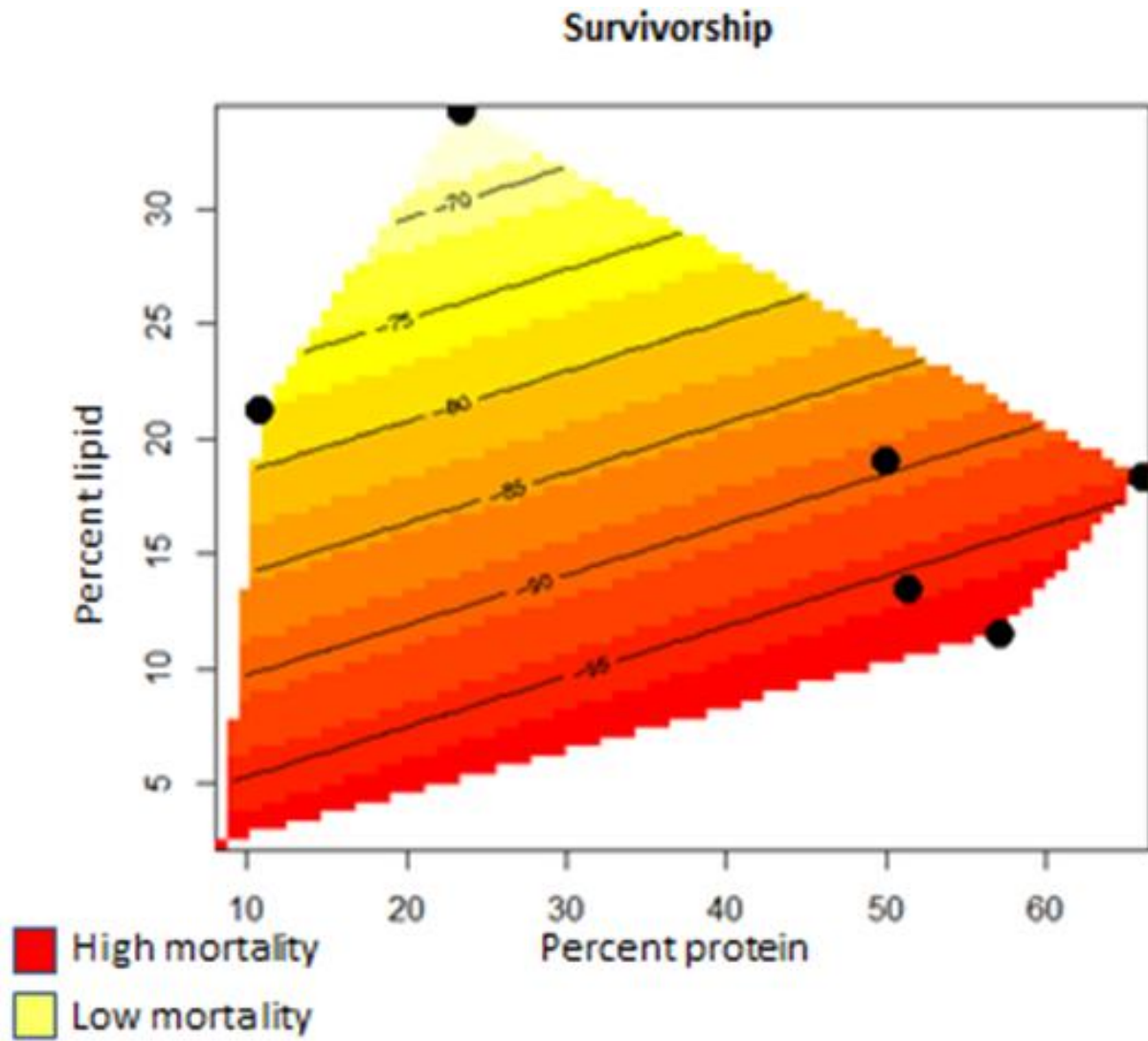
In order to better visualize the effects of diets on colonoid performance, Figs. 12-14 illustrate the expected performance of *N. fulva* colonoids when fed diets of varying protein: lipid concentrations.



**Fig 12.** Heat map of brood production within the nutritional geometric framework with respect to *N. fulva* brood production. The lighter areas denote increased brood production, while the darker areas denote decreased brood production based on diet protein to lipid ratios.



**Fig. 13.** Heat map of weight loss experienced by colonoids within the nutritional framework. The lighter areas denote a smaller loss of weight, while the darker areas denote a larger loss of weight as a result of diet protein to lipid ratio.



**Fig 14.** Heat map of worker survivorship within the nutritional geometric framework. The lighter areas denote less mortality experienced, while the darker areas denote higher mortality due to the diet's protein to lipid ratio.

## Discussion

In this study, we attempted to identify an insect-based diet that is suitable for long term rearing of *Nylanderia fulva* colonies in a lab setting. In order for colonies of any species to survive and reproduce, they require a range of micro and macronutrients in the proper balance. Even if all necessary nutrients are present in a diet, the colonies may suffer if the ratio of nutrients is imbalanced (Cook et al. 2010, Boersma and Elser 2006). Without a balanced source of nutrition, colony members may not be able to carry out physiological processes. Workers rely on carbohydrates as an energy source to accomplish tasks necessary for colony maintenance such as foraging, while reproductive members such as queens and developing larvae require higher protein levels in their diet (Cook et al. 2010). Because colonies contain many members with differing nutritional requirements, finding a single diet which will satisfy the needs of every colony member is challenging.

When the lipid and protein concentrations of the diets were regressed, it was discovered that the ratio of these two macronutrients present in the selected diets formed two rails in the nutritional geometric framework; one rail containing low protein and high lipid (beet armyworm and mealworm), and the other rail containing high protein and low lipid diets (*Drosophila*, cricket, and termite) with the artificial diet serving as the origin of these two rails. The use of the geometric framework allowed us to visualize these two nutritional rails and to infer the effects of protein and lipid concentrations on the ant colonies.

To quantify nutrient regulation on the colony level, we measured the mass of diet removed by the ants after every 48 hour feeding period. With the exception of mealworms, our results revealed that ants fed less on diets with high protein levels. This indicates that the colonies were receiving an excess of protein, thus attempting to regulate their nutrient intake.

Excess protein in diets can be toxic in insects (Boersma and Elser 2006). An overabundance of protein in the diet can result in gluconeogenesis. This physiological process lowers lipid reserves, resulting in leaner ants which experience higher mortality rates (Cook et al. 2010, Arganda et al. 2017). Typically, ants fed on a single high protein/low carbohydrate diet increase their foraging activity to compensate for the shortage of carbohydrates necessary for worker activity, indicating that foraging behavior may be strongly tied to carbohydrate deficiency in these scenarios (Dussutour and Simpson 2009). However our colonies did not experience a lack of carbohydrates due to the presence of a sucrose solution. When carbohydrate requirements were met and the only available diet was high in protein, the colonies seemingly reduced their consumption rate. The cause of the low rate of consumption of mealworms is not known, although it may be due to defensive compounds or some other unpalatable substance present in the mealworm's tissues. These compounds may have been released during the homogenization step in the diet preparation, as the larvae of some Tenebrionidae species are known to secrete defensive compounds when highly disturbed (Attygalle et al. 1991, Dettner 1993).

As a measure of overall colony health, worker mortality was tallied every 48 hour feeding period. Our results show that colonies fed no diet, meaning they were only provisioned with water and sugar water, experienced the highest rate of mortality. All other diets experienced lower rates of mortality indicating that any diet, even with poor nutrient composition, is more beneficial to colony health than no source of nutrition. According to our findings, colonies had a high rate of survivorship when fed lipid rich diets, and experienced high mortality when fed protein rich diets. This seemingly toxic effect of proteinaceous diets corroborates our findings when we examined the change in colony weights at the end of the study. Proteinaceous diets cause a greater decline in colony weight, while lipid rich diets reduced colony weight-loss.



Interestingly, the relationship between protein concentration and change in colony weight is more tightly correlated than protein content and survivorship. The high mortality experienced by colonies fed protein rich diets is consistent with other studies in ant nutrition (Dussutour and Simpson, 2012). This phenomenon is not unique to ants, and could again be explained by the costly physiological coping processes associated with exposure to diets too high in protein (Cook et al. 2010, Hamilton et al. 1990, Lee et al. 2008). Because protein rich diets can deplete somatic lipid stores in insects, the colony members that do survive may be leaner and thus weigh less, reducing the overall mass of the colony (Cook et al. 2010, Arganda et al. 2017).

The goal of this study was to determine a diet able to sustain *N. fulva* colonies in a lab over the long term. Reproductive rates in the ant colony are important to colony longevity. In order to quantify reproductive health in the colony, the weight of brood produced was recorded at the conclusion of the study. Interestingly, all colonies produced some measurable amount of brood; even those supplied only water and sugar water. Although protein is essential for both reproduction and growth of developing larvae, diets with the highest protein concentration produced the least amount of brood over the course of the study. Again this may be attributed to the physiologically costly effects of high protein diets (Cook et al. 2010, Hamilton et al. 1990, Lee et al. 2008). Although protein rich diets did produce brood, they were vastly outclassed by colonies fed beet armyworms. Because this diet was so low in protein content, ants could forage on it freely while avoiding the negative effects of a high protein diet. This may have enabled them to meet other nutritional demands while still maintaining healthy levels of protein.

Although higher in lipid content, mealworms also possessed relatively low levels of protein. If the ants had found mealworms more palatable, they may have experienced levels of reproductive success similar to colonies fed beet armyworms. Unfortunately, beet armyworms are an invasive

species and require an APHIS permit to obtain, which may be difficult for some labs to acquire. Thus determining a suitable replacement with similar nutritional content should be explored. Because colonies were collected from the environment, the replications which were denied any diet likely utilized their somatic stores of nutrients to produce their brood. In the field, *N. fulva* colonies are able to rapidly grow and outcompete local ant populations, indicating these invasive colonies are receiving adequate nutrition. Gut content analysis could be utilized to determine what these colonies are consuming. This would allow us to better understand and emulate a suitable diet for long term rearing of *N. fulva* in a lab setting

Although all colonies successfully produced brood, they also uniformly declined in weight over the course of the study if only fed a single diet. This signified to us that none of the diets we examined when fed to *N. fulva* colonies alone were sufficient to establish long term lab colonies. In order to determine a diet that would be suitable for rearing *N. fulva* colonies for long periods, we attempted to combine several of the top performing diets with the standard artificial diet. This allowed the ants to regulate their nutrient intake themselves. When supplied with more than one diet, colonies performed better than colonies only fed a single diet. This is most likely due to ant's ability to regulate their nutrient intake via selective choice (Holldobler and Wilson, 1990; Simpson and Raubenheimer, 2012). If multiple diets are present, ants may selectively forage on the diets at differing rates in an attempt to reach ideal nutrient levels. Selective foraging would also allow the colonies to avoid an excess of certain nutrients, such as protein, which can lead to harmful effects in the colony. When we supplied both an insect and artificial diet to the ant colonies, overall colony health was improved due to a reduction in mortality and an increase in brood production when compared to the single diet replications. Colonies supplied a combination of beet armyworm and the artificial diet, in addition to increased brood

production, also experienced an increase in colony weight. This increase of both brood production and weight gain suggests that ants in these colonies were able to regulate the intake of nutrients to ideal and healthy levels, resulting in colony growth.

It is my hope that these results will aid other labs rear *N. fulva* for future research. Although these results show promise, more research should be conducted into the effect of nutrition on *N. fulva* to develop a more effective diet for rearing this ant species.

## CHAPTER IV

### SUMMARY AND CONCLUSIONS

*Nylanderia fulva* is a newly introduced invasive species to coastal and central Texas. Due to the colony structure of this species, and its rapid rate of reproduction, there has been difficulty developing effective control methods in urban environments. Because of its relatively recent introduction and the lack of effective control methods, more research is needed in order to increase our understanding of this invasive species. One reason for this is *N. fulva*'s ability to displace established ant populations in the invasive range. In order to better understand this displacement, we analyzed the aggressive response of *N. fulva* in the presence of local ant species present in Central Texas, providing a possible mechanism of the displacement of large bodied ants, as observed by LeBrun et al. (2013). In order to increase the availability of *N. fulva* for scientific study, we also investigated a suitable diet to rear long term colonies of *N. fulva* in a lab setting. *N. fulva* worker abundance dramatically decreases during the cooler months of the year, and do not resurge until late spring, reducing their availability for study (personal observation).

When investigating the aggression that *N. fulva* workers display to other ant species, it was discovered that the aggressive response of *N. fulva* increased as the head capsule size of the foreign ant species increased. Although ant aggression as a function of the opponents' size or the size difference between competing species has not been heavily studied, there is some support in the literature which suggests that some ant species will alter their aggressive behaviors when faced with opponents of differing sizes (Nowbahari et al. 1999, Horn et al. 2013). *N. fulva*'s increased aggression towards ants with larger head capsule size also provide a potential

mechanism for the increased rate of displacement experienced by large bodied ant species observed in *N. fulva*'s invasive range (LeBrun et al. 2013).

Nutrition experiments revealed that *N. fulva* colonoids experienced lower worker mortality and higher brood production when fed diets that contained a low protein to lipid ratio. This study also revealed that excessive protein in the diet resulted in high *N. fulva* worker mortality, indicating a possible toxic effect of excess dietary protein. *N. fulva* colony health was also improved when supplied both the artificial diet and an insect diet, presumably due to the workers' ability to more readily regulate their nutrient intake, or the increased availability of micronutrients present in the artificial diet. This allows us, in theory, to successfully grow a colony of *N. fulva* over the course of the winter. However, this study only ran for six weeks, and more long term research is necessary to determine if the diets used in this study are suitable for year-round use in lab colonies, as nutritional demands may change over time. Although our study was focused on *N. fulva* colony health, a utilization of the geometric nutritional framework of diets, along with a suitable quantitative measure of health (such as brood production, change in colonoid weight, and worker mortality, as used in this study), appropriate diets may be investigated for any species of interest.

The lack of knowledge regarding *N. fulva* is troubling. We do not fully understand its colony structure, method of reproduction, or the long term consequences of its presence in the environment. Therefore it is important that viable rearing protocols be developed, so that our ability to study this important invasive species is enhanced, which may lead to a more complete understanding of *N. fulva*'s ecological effects, as well as development of new potential control methods.

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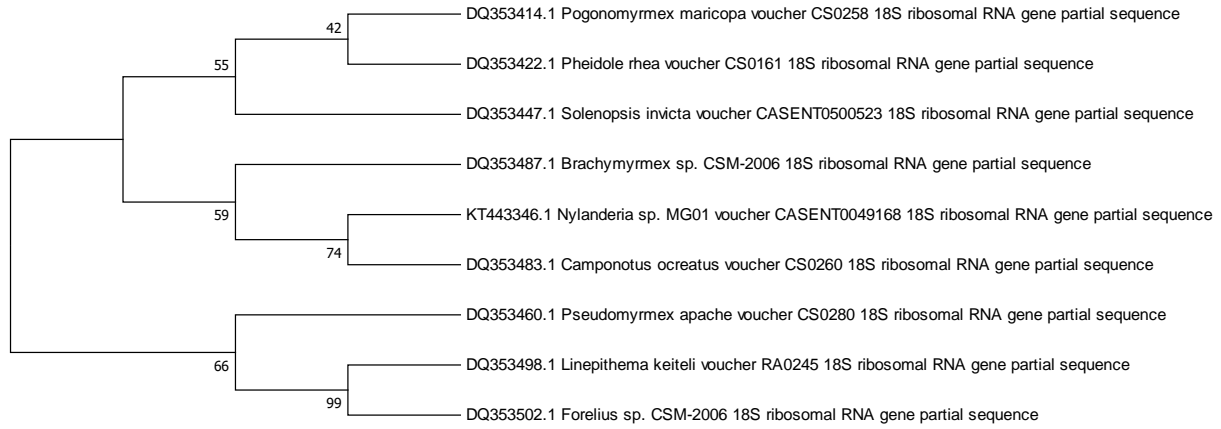


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## APPENDIX



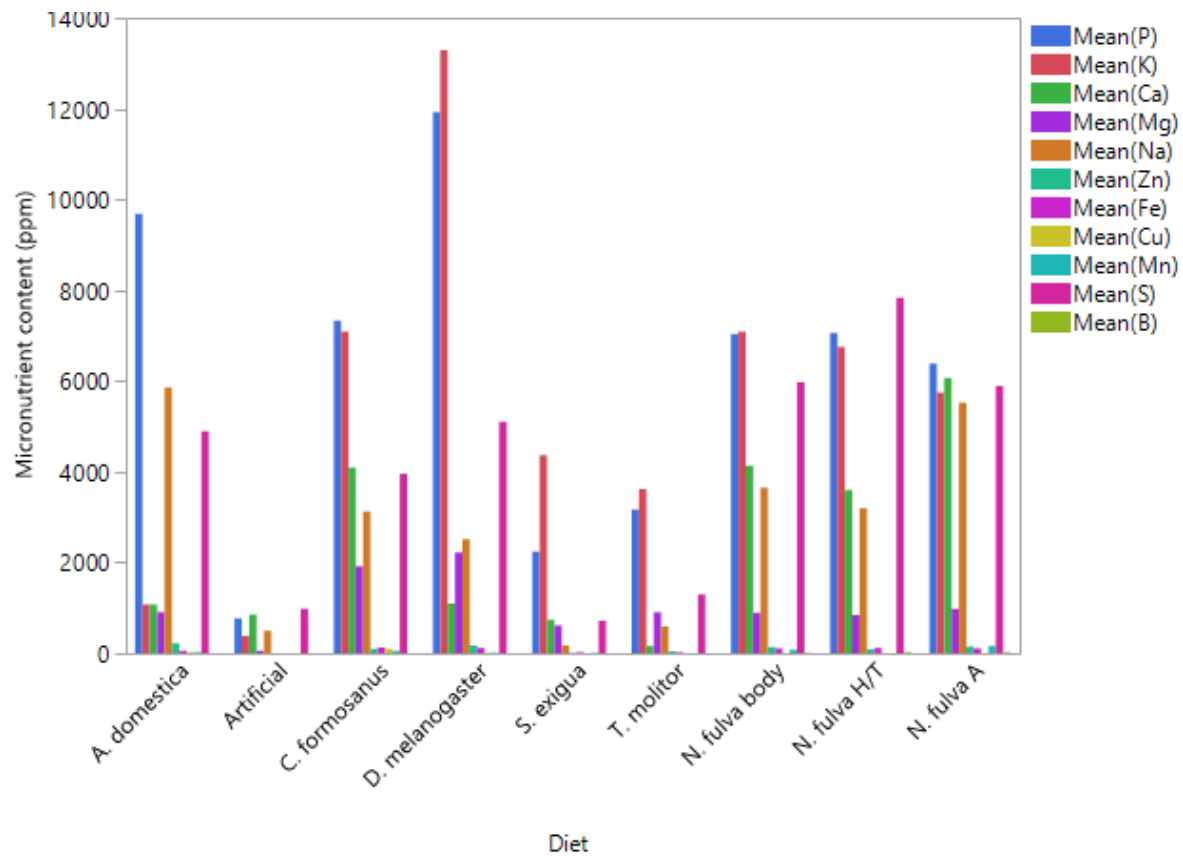
**Fig. A-1.** Evolutionary relationships of taxa based on 18s sequences. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.04591464 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 657 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

**Table A-1.** Pairwise base differences per sequence. Standard error estimate(s) are shown above the diagonal. The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 657 positions in the final dataset. Evolutionary analyses were conducted in MEGA

Species	1	2	3	4	5	6	7	8	9
1 <i>Solenopsis_invicta</i>		3.702	2.811	2.811	2.632	3.951	3.951	2.632	2.438
2 <i>Pseudomyrmex_apache</i>	14		3.138	3.57	2.979	3.57	3.702	2.979	2.811
3 <i>Pogonomyrmex_sp.</i>	8	10		2.228	2.228	3.57	3.702	2.228	1.994
4 <i>Pheidole_rhea</i>	8	13	5		2.438	3.432	3.57	2.438	2.228
5 <i>Nylanderia_sp.</i>	7	9	5	6		3.289	3.138	0	0.999
6 <i>Linepithema_keiteli</i>	16	13	13	12	11		1.728	3.289	3.432
7 <i>Forelius_sp.</i>	16	14	14	13	10	3		3.138	3.289
8 <i>Camponotus_ocreatus</i>	7	9	5	6	0	11	10		0.999
9 <i>Brachymyrmex_sp.</i>	6	8	4	5	1	12	11	1	

**Table A-2.** List of all possible models explaining *N. fulva* aggression when encountering competing ant species in central Texas.

Model	R square	AIC
1 Size	0.3314	287.205
2 Phylogenetic distance	0.0038	247.491
3 Historic interaction	0.333	249.342
4 Size*Phylogenetic distance	0.4527	230.937
5 Size*Historic Interaction	0.4852	226.811
6 Phylogenetic distance*Historic interaction	N/A	N/A
7 Size*Phylogenetic distance*Historic interaction	N/A	N/A

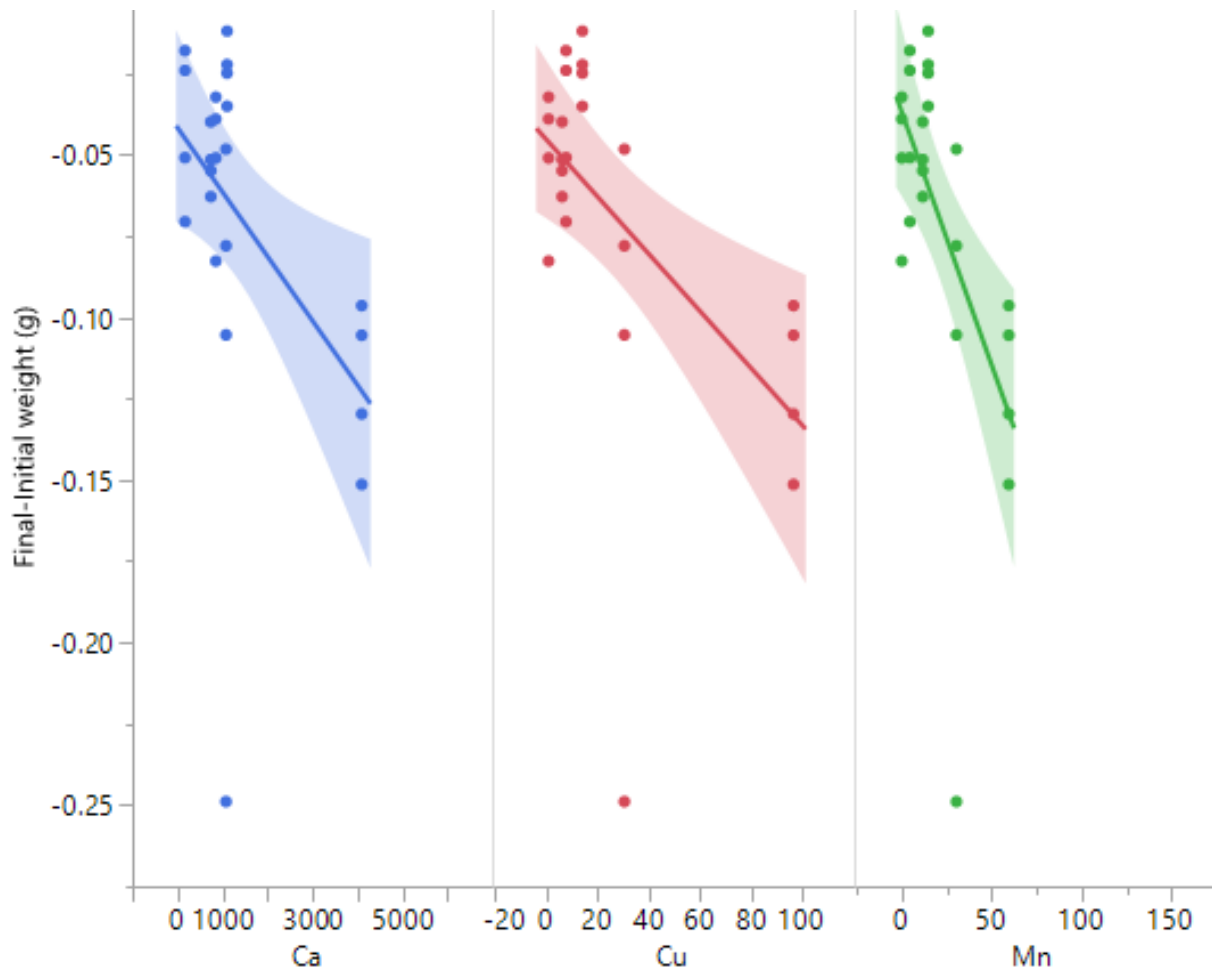


**Fig. A-2.** Micronutrient content of diets and *N. fulva* full body, head/thorax, and abdomen. Values in the analysis are given in parts per million.

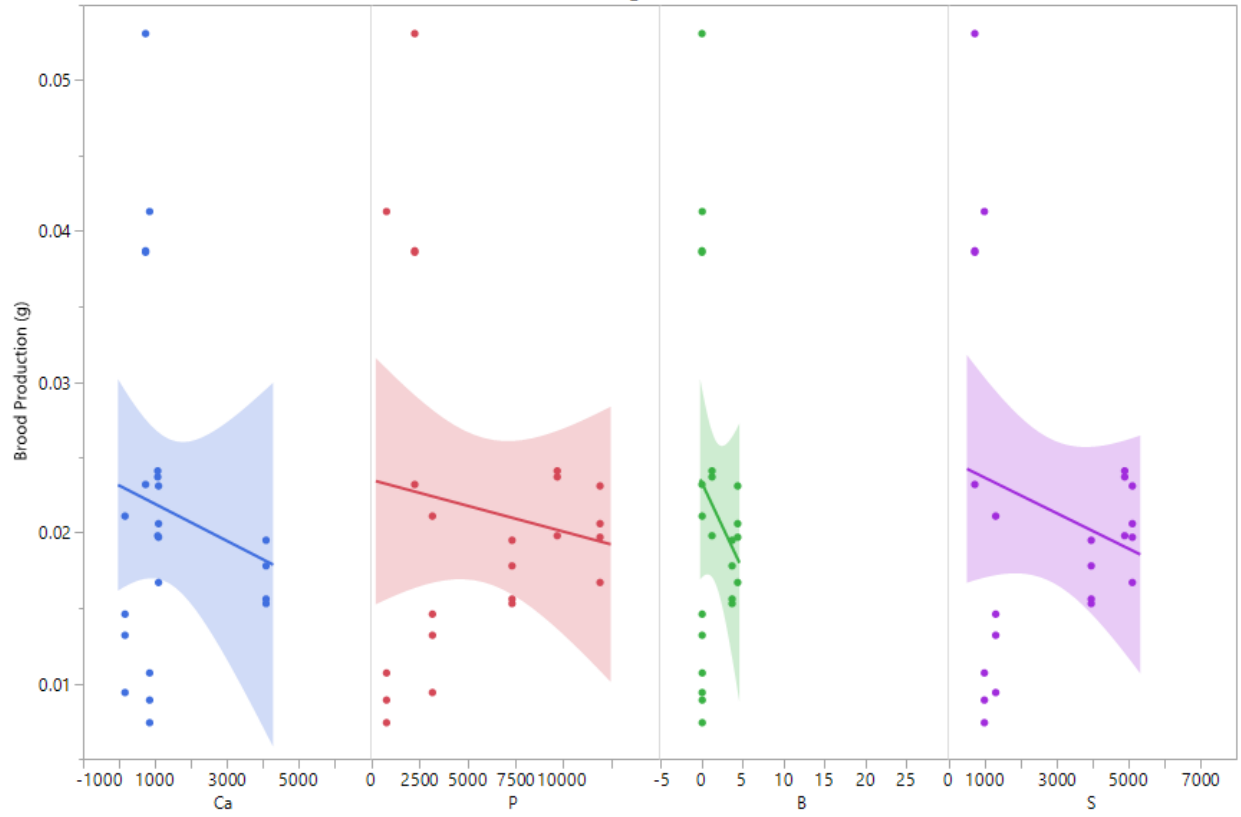
**Table A-3.** Macro and micronutrient analysis of all diets fed to *N. fuhva* colonoids. Values are given in ppm unless otherwise noted.

Species	Lipid (%)	Protein (%)	Water (%)	Protein : Lipid	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S	B
<i>C. formosanus</i>	11.51	57.179	61.3	4.967767159	7327.8	7091	4097	1919	3132	95.2	130	96.3	59.6	3961	3.74
<i>A. domestica</i>	18.34	66.157	74.8	3.607251908	9690	1079	1079	908	5863	224	59.9	30.5	30.5	4893	1.29
Artificial	2.31	8.42	71.8	3.645021645	771.12	388.68	852.9	61.24	494.22	4.42	6.02	1.02	0.11	984.68	0.1
<i>T. molitor</i>	34.31	23.47	54.3	0.684057126	3169.3	3625.1	167.49	912.6	593.23	43.7	21.3	7.8	4.59	1300	0.09
<i>S. exigua</i>	21.27	10.89	78.5	0.511988717	2245.2	4367.3	740.03	619.6	174.72	17.8	22.6	6.28	11.8	717.72	0.07
<i>D. melanogaster</i>	13.4	51.413	67.3	3.836791045	11924	13295	1102	2228	2513	176	123	14.2	14.8	5107	4.42





**Fig. A-3.** Micronutrient correlation with weight loss. As calcium, copper, and manganese increase in the diet, the overall weight loss experienced by colonoids increases.



**Fig. A-4.** Micronutrient correlation with brood production. As micronutrient content increases overall brood production decreases