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Using Behavioral Experimentation to Understand the Social Structure of the Little Fire Ant (*Wasmannia auropunctata)* in Florida

Jacob Aron Hornfeldt, Leo Ohyama, & Andrea Lucky
University of Florida

Faculty Mentor: Andrea Lucky, Department of Entomology & Nematology

Abstract

Wasmannia auropunctata (little fire ant: LFA) is a small yellow ant that is native to Central and South America and has become a globally invasive species by acting as an agricultural pest in addition to being ecologically destructive in non-native regions. One trait thought to contribute to successful invasion in ants is unicoloniality, which is characterized by lack of aggression among workers and queens from different colonies. This results in colonies becoming effectively borderless, expanding as a single, massive colony across a large geographic distance. These ants are known to be unicolonial in some parts of their range, but no studies to date have assessed unicoloniality in Florida populations. To determine whether LFA populations across Florida were members of a unicolonial lineage, we carried out integration trials using 27 colonies from six sites in three Florida counties. Approximately 20 workers from different colonies were placed together in an arena; after 24 hours they were evaluated to determine whether or not they integrated. Out of a total of 42 trials, 17 (40%) resulted in full integration and 25 (60%) did not. Colonies that were collected within 10 km of each other integrated, and ants from colonies beyond that distance did not integrate. These results suggest that the little fire is not unicolonial in Florida on a large scale, but perhaps on a smaller scale. Future studies should attempt to use colonies from more counties and incorporate genetic data to determine relatedness among these ants.

Keywords: little fire ant, social structure, unicoloniality, behavior, invasive, Florida

Introduction

Wasmannia auropunctata (Roger) (the little fire ant: LFA) is a small, yellow ant native to Central and South America that has become a globally invasive species. It has become established in tropical and subtropical regions of the world including parts of the United States, Africa, Europe, Australia, and the Pacific Islands (Wetterer, 2013). In areas where the LFA is non-native, it has become a serious pest that threatens native biodiversity because of its ability to outcompete native arthropod fauna (Le Breton et al., 2003). While research on these exact impacts are limited, a study in New Caledonia found that when little fire ants are present, they reduced ant species richness and overall ant abundance (Le Breton et al., 2003). The little fire ant also causes significant economic loses related to agriculture, livestock, tourism and more. In

Hawaii alone, it is estimated that this species results in the loss of $549 million annually (Lee et al., 2015). Because of the ecological and economic significance of this ant, it is important to understand how it might potentially be spreading in invaded regions, like Florida. The LFA social structure is expected to influence its ability to spread rapidly, particularly in non-native regions.

One trait possessed by many successful invasive ants—including the LFA—is unicoloniality, a social structure in which workers and queens exhibit a lack of aggressiveness towards closely related non-nest mates of the same species, resulting in free movement between different nests (Eyer et al., 2018). This allows for a single supercolony to expand, unlimited by colony borders, across large geographic distances, which can lead to a high density of workers, queens, and brood that allows for increased colony survival (Eyer et al., 2018; Holway et al., 1998). Invasive ants that are known to form supercolonies include argentine ants (*Linepithema humile* (Mayr)), tropical fire ants (*Solenopsis geminata* (Fabricius)), and yellow crazy ants (*Anoplolepis gracilipes* (Smith, F.)), among others (Helantera et al., 2009). Greater colony success, including faster growth and reproduction, higher worker density, and larger colony size, can make it more difficult for humans to effectively control and manage ant pests (Silverman & Brightwell, 2007). Increasing our understanding of unicolonial ants can help to inform management and monitoring techniques, which can in turn aid conservation efforts and help to reduce economic losses.

LFA is unicolonial in its non-native and parts of its native range, though not all unicolonial populations can integrate together as a single colony (Le Breton et al., 2004). Rather, in many places individual colonies have the ability to form supercolonies but remain distinct from and aggressive towards other colonies (Le Breton et al., 2004). In some cases one supercolony has become very large and dominates an entire area (Le Breton et al., 2004). For example, using behavioral assays, Le Breton et al. (2004) demonstrated LFA unicoloniality in the introduced range of New Caledonia and in the native range of Brazil. In New Caledonia, this unicoloniality was at a large scale, as ants up to 450 km apart showed little to no aggression toward one another other, while the Brazilian unicolonial populations were limited to a smaller scale, generally less than 3 km apart. This study also noted that within its native range the LFA appears to be more multicolonial as opposed to unicolonial, which means that ants of the same species form discrete bounds (Le Breton et al., 2004). This has also been documented in other invasive ant species, where unicoloniality is common in the non-native range, but occurs more rarely in the native

regions, such as with tawny crazy ants and argentine ants (Eyer et al., 2018; Holway et al., 1998). In tawny crazy ants (*Nylanderia fulva* (Mayr)) this pattern may be due to a genetic bottleneck following an introduction event (Eyer et al. (2018). This might suggest that unicoloniality follows patterns of introduction events into a non-native region where a single introduction might result in unicoloniality because of reduced genetic variation among introduced individuals, whereas multiple introductions might result in reduced levels of unicoloniality because there is more genetic variation present. Understanding unicoloniality could therefore be useful as a proxy for deducing the introduction history of an ant.

Few studies have explored the behavior of the LFA worldwide, and no studies to date have explored unicoloniality using behavioral assays in Florida. The goal of this study is to determine if unicoloniality of the LFA exists in Florida, and if so, to what extent.To answer this question, this study implemented behavioral trials between colonies of LFA that utilize integration, whereby many workers interact with and show no aggressive behavior towards many workers of another colony, as a method to determine unicoloniality.

The results of this study will help clarify the social structure of the LFA populations in Florida. In turn, these results can potentially be used to improve understanding of the invasive history of the LFA in this region. If all populations sampled are found to integrate, then the ant may be acting as a single, large colony throughout the state, similarly to the population in New Caledonia. This might point to a single introduction of the species into the state. By contrast, if some populations integrate and some populations do not, then the social structure may be more similar to the population in Brazil. This may suggest multiple and even possibly ongoing introductions.

Materials and Methods

Collection Sites

Colonies of LFA were collected from three sites in Broward County, two sites in Alachua County, and one site in Palm Beach County for a total of 27 colonies from the six sites (Table 1; Figure 1). The minimum distance between sites was 2 km and the maximum distance was 454 km. These locations were chosen to reflect similar distances seen in Le Breton et al. (2004) where lack of aggression was seen across these large distances suggesting large scale unicolonality.

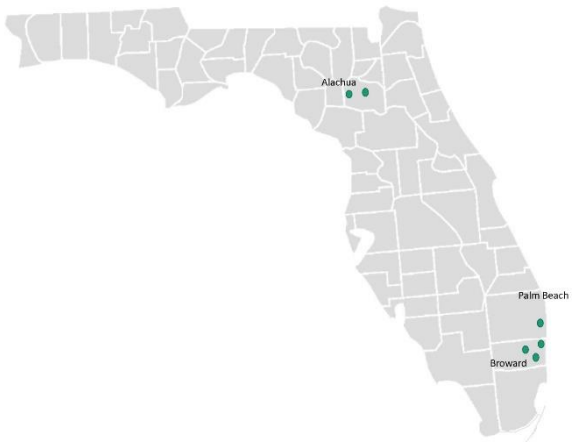

Figure 1. Map showing rough location of colony collection sites. Image courtesy of Wikimedia Commons.

Table 1. Collection Information for Each Colony.

Colony	Lat	Long	Location Name	City	County	Date
AC-1.6	29.6447	-82.3623	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.7	29.6446	-82.3616	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.8	29.6445	-82.3617	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.9	29.6448	-82.3624	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.10	29.644635	-82.36184	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.11	29.644635	-82.36184	UF Campus: Field and Fork Garden	Gainesville	Alachua	19-Oct-19
AC-1.12	29.644762	-82.36161	UF Campus: Field and Fork Garden	Gainesville	Alachua	1-Nov-19
AC-1.13	29.644736	-82.36171	UF Campus: Field and Fork Garden	Gainesville	Alachua	18-Jan-20
AC-1.14	29.644736	-82.36171	UF Campus: Field and Fork Garden	Gainesville	Alachua	18-Jan-20
AC-2.7	29.66333	-82.40055	Brentwood School	Gainesville	Alachua	18-Jan-20
AC-2.8	29.66333	-82.40055	Brentwood School	Gainesville	Alachua	18-Jan-20
AC-2.9	29.66333	-82.40002	Brentwood School	Gainesville	Alachua	18-Jan-20
BC-1.1	26.152587	-80.10539	Hugh Taylor Birch State Recreation meditation garden	Ft. Lauderdale	Broward	27-Oct-19
BC-2.1	26.088828	-80.17699	Secretwoods Pollinator Garden	Ft. Lauderdale	Broward	27-Oct-19
BC-2.2	26.088805	-80.17685	Secretwoods Pollinator Gardens	Ft. Lauderdale	Broward	27-Oct-19
BC-2.3	26.089633	-80.17709	Secretwoods Laurel Oak Trail	Ft. Lauderdale	Broward	27-Oct-19
BC-2.4	26.0891	-80.1769	Secretwoods Laurel Oak Trail	Ft. Lauderdale	Broward	2-Jan-20
BC-2.5	26.0891	-80.1769	Secretwoods Laurel Oak Trail	Ft. Lauderdale	Broward	2-Jan-20
BC-2.7	26.0887	-80.1681	Secretwoods Pollinator Garden	Ft. Lauderdale	Broward	2-Jan-20
BC-3.1	26.172542	-80.16217	Easterlin Park	Ft. Lauderdale	Broward	27-Oct-19
BC-3.2	26.172435	-80.16216	Easterlin Park	Ft. Lauderdale	Broward	27-Oct-19
BC-3.4	26.172	-80.1622	Easterlin Park	Ft. Lauderdale	Broward	2-Jan-20
BC-3.5	26.172	-80.1622	Easterlin Park	Ft. Lauderdale	Broward	2-Jan-20
BC-3.6	26.1723	-80.1612	Easterlin Park	Ft. Lauderdale	Broward	2-Jan-20
PC-1.1	26.667615	-80.16811	Okeeheelee park nature center	West Palm Beach	Palm Beach	28-Oct-19
PC-1.2	26.6676	-80.1681	Okeeheelee park nature center	West Palm Beach	Palm Beach	2-Jan-20
PC-1.3	26.6676	-80.1682	Okeeheelee park nature center	West Palm Beach	Palm Beach	2-Jan-20

Integration Trials

To test for unicoloniality, 42 integration trials were run using the 27 colonies. Colonies were haphazardly paired to maximize the number of times that two sites were placed together. About 20 workers from two different colonies were placed together for 24 hours in an arena, a 4 cm x 12 cm x 5 cm container with fluon-coated walls and air flow holes in the lid. Workers were differentiated by painting individual ants with enamel paint prior to the start of the trial (Figure 2). Following the 24-hour period, the ants were evaluated to determine if integration had occurred. Integration was defined as workers from the two colonies clumping together and freely interacting with one another with no aggression (Figure 3A). Lack of integration was determined by two distinct clumps of workers (painted and non-painted) and/or if the workers from the different colonies were observed engaging in aggressive behavior (e.g., biting at each other, stinging, etc.) (Figure 3B).

Figure 2. Little fire ant with a drop of green paint used to differentiate it from another colony. Photo courtesy of V.R. Seagal.

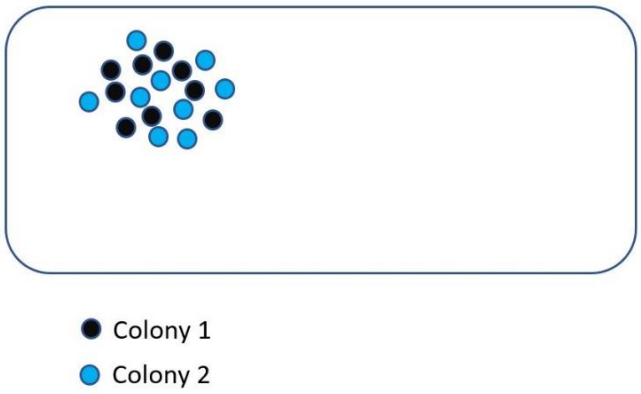

Figure 3A. Expected outcome of behavioral trial when ants integrate, suggesting unicoloniality. (Not to scale)

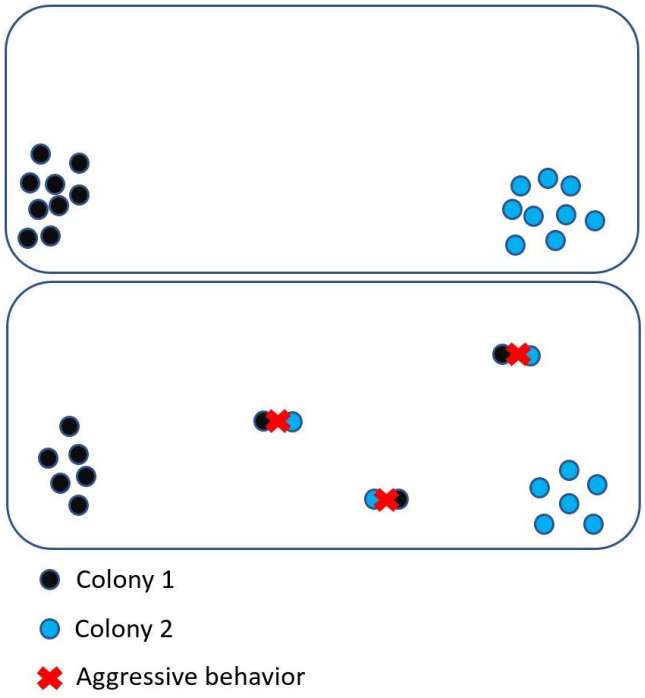

Figure 3B. Expected outcome of behavioral trial in the absence of integration, suggesting lack of unicoloniality. (Not to scale)

Results

Of 42 total integration trials, 17 pairings resulted in integration (40%) and 25 did not (60%), (Figure 4, Table 2). Each site pairing had consistent behavior, with sites that integrated once consistently integrating and the same being true for non-integration. The 17 pairings that did integrated were from sites no more than 10 km apart, while the 25 sites that did not integrated were from sites farther than 10 km apart. (Figure 5).

Figure 4. Integration trial results. Each line represents a behavioral trial: blue lines represent integration and gray lines represent no integration.

Table 2. Integration trial pairings and results.

Site name	AC-1	AC-2	PC-1	BC-1	BC-2	BC-3
AC-1	**3** (0)	**3** (4)	**2** (394)	**x**	**4** (448)	**3** (441)
AC-2		**2** (0)	**1** (397)	**X**	**3** (452)	**3** (445)
PC-1			**X**	**1** (57)	**3** (64)	**2** (55)
BC-1				**X**	**3** (10)	**2** (6)
BC-2					**2** (0)	**3** (9)
BC-3						**2** (0)

Note: Numbers represent the number of times a site was paired together in trials, the number in parentheses indicate the distance (rounded to nearest km) between the sites. X indicates that no trials were run between sites. Blue cells indicate that integration always occurred, white cells indicate that integration never occurred.

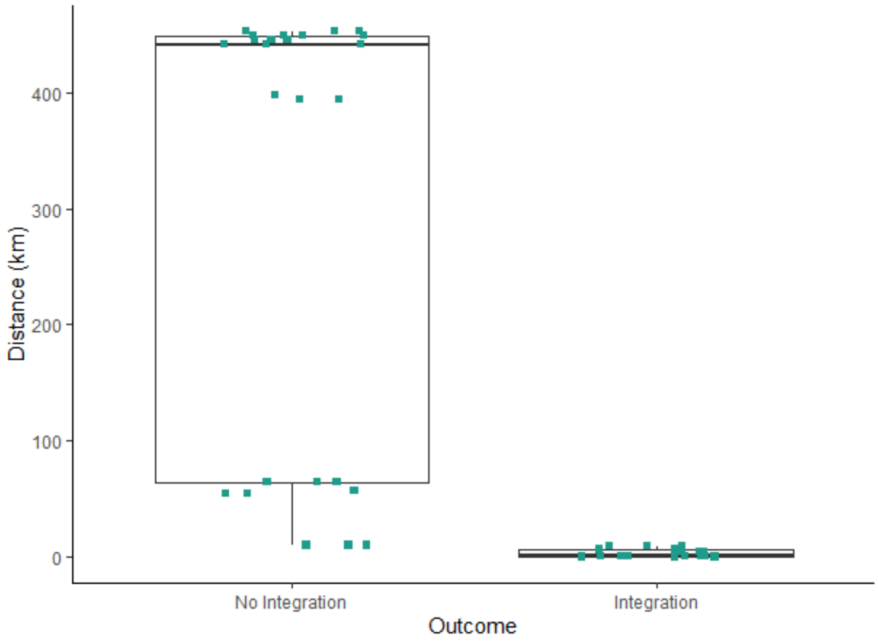

Figure 5. Boxplot showing integration trial outcomes as they varied by distance

Discussion

Behavioral integration trials revealed unicoloniality of LFA in Florida, but lack of integration across distances greater than 10 km suggest limitations to the scale of unicoloniality throughout the state. Because 10 km acted as a distance threshold by which colonies integrated in this study, this may suggest that distance is a useful tool for deducing the number of distinct unicolonial populations, though it may also be an artifact of limited sampling. The colonies that did integrate were all closer than 10 km apart, and their integrations may reflect either expansion of a single colony or possible human-mediated transport of ants between sites (e.g., through movement of plants or landscaping materials). As distance between colonies increases and there is less flow of

workers, ants may see a reduction in unicoloniality, though this is unclear. Human mediated movement of ants may be significant in terms of the genetic relatedness of ants between sites, which can influence the intraspecific aggression of the ants seen there (Van Wilgenburg et al., 2010).

Genetic relatedness of LFApopulations is likely an important factor to take into consideration when exploring social structure, because closely related ants may be more likely to exhibit a lack of aggression toward eachother than ants that are not closely related (Van Wilgenburg et al., 2010). Mikheyev & Mueller (2007) suggest that LFA lineages can be split into two main clades, with multiple introductions into non-native ranges occurring from each of these clades. Even within these clades, individuals from different introductions may be from separate genetic lineages, which then could have resulted in two or more distinct lineages within Florida. This may be the case, considering all populations sampled did not integrate with each other. Distinct colony boundaries have been documented in other unicolonial ants; Helantera et al. (2009) shows that in several other species there is an observable genetic distinction between two different supercolonies of the same species. This supports the idea that Florida may have more than one unicolonial lineage of the LFA .

These results provide insight into the unicoloniality of LFA in Florida. Despite the small sample size employed in this study, we can infer that there may have been multiple introductions of the LFA into the state, since we did not see large scale integration but rather smaller pockets of it within north and south Florida. This can also help to inform control methods of the LFA in the state. Since they do not seem to exhibit large scale unicoloniality, control efforts might use this information to be able to effectively target smaller populations and prevent spread of these smaller unicolonial pockets.

It is important to note that this study was limited in the number of sites used and the number of colonies collected and using more sites from different locations (e.g., central or south west Florida) may have revealed a different pattern of LFA social structure in the state. As such, future studies might benefit from including more sites from more locations throughout Florida. Additionally, because genetic tools were not used to determine relatedness, future studies would benefit from incorporating genetic data with behavioral data. Research should continue to explore LFA social structure to gain a better understanding of the ant, its ecological and economic impact, and how it can be monitored and managed.

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