Agonistic Interactions Among Invasive Ant Species (Hymenoptera: Formicidae) from Two Habitats on Oahu, Hawaii

by

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ABSTRACT

The Hawaiian Islands represent the most isolated land mass in the world, with no native ant species. To study invasive biology within two ant communities on Oahu, Hawaii we conducted laboratory bioassays to assess agonistic interactions among five introduced ant species: *Leptogenys falcigera* (Roger,), *Camponotus variegatus* (F. Smith), *Anoplolepis gracilipes* (F. Smith), *Ochetellus glaber* (Mayr), and *Pheidole megacephala* (Fabricius). Both individual and group assays were conducted, pairing either individual ant workers or groups of ten workers from each species. Individual assays were scored using a rating system ranging from aggressive to defensive behavior. Group assays were evaluated on the basis of ant mortality, assuming that aggression led to mortality of one or both of the species. The species with the highest and lowest levels of agonistic behavior were similar in individual and group experiments. *Pheidole megacephala* ranked first with the highest level of aggression in the individual assays and highest mortality in group assays. *Anoplolepis gracilipes* ranked second in aggressive behavior in individual assays and second in group assays. *Leptogenys falcigera* was the most non-responsive in individual assays, and had 100% survival in the group trials. These experimental results correspond with our field observations that *P. megacephala* and *A. gracilipes* are numerically dominant species and *L. falcigera* is a subdominant species on Oahu, Hawaii.

Key Words: Invasion biology, ants, agonistic interactions, laboratory assays, Hawaii

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INTRODUCTION

The Hawaiian Islands are the most isolated archipelago in the world. These islands have a complex assemblage of plants and endemic arthropods that evolved without any Formicidae predators (Wilson & Taylor 1967). A small subset of the 45 exotic ant species that have been successfully established in Hawaii are considered to be tramp ants (i.e. polygynous, generalist, and with a large colony size) (Holway *et al.* 2002). With respect to which species will become dominant in the invaded habitat, previous research has proposed that a predictive tool would be useful to protect ecosystems from destructive alien species (Holway *et al.* 1998, Holway *et al.* 2002, Holway & Suarez 1999, Moller 1996, Morrison 2000a). We suggest that agonism among invasive ant species in Hawaii can be used as a tool in predicting which species is the most dominant with the potential to have greater ecological effects on native arthropods and plants.

A study conducted by Morrison (1996) in the Society Islands, French Polynesia also examined the invasive biology of ants. These islands are similar to the Hawaiian Islands, in that they also lacked native ants. Morrison's research assessed the community structure of dominant and subdominant ant species, particularly how non-native ant communities establish when introduced to an insular ecosystem. Morrison (1996) found that one mechanism leading to the dominance of invasive *Solenopsis invicta* is its superior ability for colony-level competition (high aggression) and cohabitation with subdominant ant species. The relationships among dominant and subdominant ant species have been studied in a number of habitats, including cocoa plantations in Brazil and Papua New Guinea (Room 1975, Majer *et al.* 1994). Many of the ant species studied have invaded Hawaii (i.e. *Anoplolepis gracilipes* [F. Smith], *Wasmannia auropunctata* [Roger] and *Pheidole megacephala* [Fabricius]), providing an opportunity to explore the community structure of invasive dominant and subdominant ant species and determine if it is consistent with patterns demonstrated elsewhere.

Interspecific competition is one mechanism that results in aggression between species. The resulting contest will often result in one species having dominance over another in a particular habitat (Hölldobler & Wilson 1990a). Holway *et al.* (2002) described the general characteristics of invasive ants, and aggression is a significant characteristic of successful species. Holway *et al.* (2002a) and Hölldobler & Wilson (1990a) report additional characteristics that are inclusive but not exclusive for all established introduced ants. These characteristics include polygynous colonies, habitat generalists, and large colony size. These characteristics are known to be important traits for successful dominant ant species, but can be confounding when studying tramp ant species. As mentioned above, tramp ants tend to have low intraspecific aggression (leading to large colonies with multiple queens) which can then lead to overlapping nests or supercolonies (Passera 1994, Hölldobler & Wilson 1977), thus making it difficult to differentiate distinct colonies in the field

Agonistic behavior, as defined by King (1973), "includes all behaviors associated with the contest or struggle between individuals." For agonistic trials in the laboratory, we sampled two different habitats on Oahu, Hawaii, in order to compare interspecific interactions of ants within and among the communities. We selected both abundant and rare ant species for this study. *Leptogenys falcigera* is an Old World species originating from Africa and one of the original ants to colonize the Hawaiian Islands in the late 1800's (Smith 1879). This nomadic ponerine ant forms small colonies (50-100 workers), has ergatoid (worker-like) queens, feeds mainly on isopods, and inhabits dry soil and tree cavities (Bolton 1973). *Camponotus variegatus* is native to Southeastern Asia and is also documented as one of the pioneering ant species in the Hawaiian Islands (Smith 1879). This ant produces colonies with approximately 100 workers, soldiers, and one queen, and feeds on sugar-based food and dead insects. The exact origin of *A. gracilipes* is not known but is speculated to be West Africa, India or China (Wetterer 2005). This species has a widespread distribution on all the major Hawaiian Islands (Reimer et al. 1990). *Anoplolepis gracilipes* is known as a tramp ant and is on the list of "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Specialist Group (ISSG 2006). *Ochetellus glaber* was first recorded on the Hawaiian Islands in 1977, and originated from Australia and Japan (Beardsley 1980). This species is abundant on Kauai, Oahu, Maui and the Island of Hawaii (Reimer *et al.* 1990). *Pheidole megacephala* is a tramp ant and is also listed on "100 of the World's Worst Invasive Alien Species" (ISSG 2006). This species was first discovered in Hawaii in 1879 (Smith 1879), and originates from southern Africa.

Our goal was to elucidate the invasive biology of the ants and gain insight into the community structure of these species in Hawaii. Combining individual and colony level behavioral assays provides a comprehensive method to test ant species agonism (Moller 1996, Retana & Cerda 1995). We addressed the following key-questions: how behaviorally dominant and subdominant species interact in terms of aggression when paired individually and in larger groups; the differences in aggression between the two types of assays; and how agonistic behavior may relate to the status of being a numerically dominant or subdominant species in Hawaii.

MATERIALS AND METHODS

Collection of ants

Ants were collected at two sites on Oahu, Hawaii that differed in elevation and temperature. The first site, Tantalus Forest Reserve above Honolulu, Hawaii, is 634 meters above sea level with an average yearly rainfall of 266.25 cm and 20.3 °C. The other site at the Waimanalo Experimental Research Station of the University of Hawaii at Manoa is on the windward side of the island of Oahu and is 18m above sea level with an average yearly rainfall of 110.76cm and an average temperature of 23.8 °C. We chose these two habitats because of their environmental and ant species variation in order to assess agonism among ant species that otherwise might not interact in the field.

Four ant species, *L. falcigera*, *C. variegatus*, *P. megacephala*, and *O. glaber,* were collected from the Waimanalo Experimental Research Station. The fifth species, *A. gracilipes*, was collected from Tantalus. To account for intraspecific behavioral variation, several colonies of each species were collected. The ants were kept in the laboratory at 25 °C and fed 25% sucrose solution, peanut butter, sweet corn, tuna fish in oil and dead insects. *Leptogenys falcigera* mainly fed on 25% sucrose solution and live isopods collected from the field.

Laboratory Bioassays

In laboratory bioassays where individuals of two species were paired, interactions were recorded using a visual rating system with a range of nine different behaviors. These assays demonstrate agonistic behavior on an individual level and can be taken as a measure of the innate aggression of each species. The second set of experiments were group agonistic assays, where groups of ten individuals from each species were paired. These experiments are more representative of agonistic interactions at the colony level.

In the individual assays, an individual of one species was gently placed with a wooden stick, or aspirated, into a 60x15 mm Petri dish coated with fluon near the rim to prevent escape. The ant was given 1-2 min to acclimate before another individual of one of the other four ant species was likewise transferred to the opposite end of the dish. We recorded any behavioral interactions for ten minutes, following the methods of Retana & Cerda (1995) with a nine-point rating system graded from offensive to defensive behavior as follows: bite, gaster flex, attack, mandibles open, indifference, mutual investigation, escape, being attacked, and being bitten. Only the minor castes were used for species with polymorphic colony structures. Each response was noted and totaled numerically, with five replicates of each species pairing. Behavioral interactions were recorded from the perspective of each of the two ant species in each trial.

Group assays were evaluated on the basis of ant mortality, with the assumption that aggressive interactions lead to mortality. Ten individuals of each species were paired in 400ml glass containers coated with fluon around the edges. *Pheidole megacephala* and *C. variegatus*, the species with polymorphic colonies, had one individual from the major caste included in group assays. Paired species were left for three hours, with four replications of each species pairing. Only one replication for pairs with *L. falcigera* was conducted due to small colony size. Controls were initiated at the same time as the two-species pairings, with 20 individuals from the same species placed in a container for three hours. At the end of the incubation period, survivors were recorded to assess mortality.

Statistical Analysis

Individual assays were analyzed by overall species behavioral response using Chi-square analysis. Specific responses within each pair of species were analyzed by GLM Procedure in SAS. T-tests were used to test mean differences in mortality among pairs in group assays (SAS Institute, 2002-2003). In the individual assays, means were separated with the Tukey's Studentized Range (HSD) Test to determine which behaviors were most frequently observed.

RESULTS AND DISCUSSION

Pheidole megacephala and *A. gracilipes* were the two species in the individual assays with the highest number of aggressive responses. Table 1 lists the total number of observations in each behavioral category (i.e. attack, bite, gaster flex, mandibles open, indifference, mutual investigation, escape, being bitten, and being attacked) when each species was paired against each of the other four species. All of the species except *C. variegatus* exhibited significant difference in frequencies of these different behaviors (Table 1.). The Chi-square values are as follows: *P. megacephala*: $\chi^2 = 146.1$; 2 df; $P < 0.05$, *A. gracilipes* $\chi^2 = 12.7$; 2 df; $P < 0.05$, *O. glaber* $\chi^2 = 33.0$; 2 df; $P < 0.05$, *L. falcigera* $\chi^2 = 326.9$, 2 df; $P < 0.05$. In Table 2, the nine behaviors are summarized into three categories: aggression, no response, and defensive; and then ranked comparatively among species. To test statistical differences among pairs we analyzed the mean of the nine behavioral interactions using the general linear models procedure (SAS Institute, 2002-2003). All pairs except the pairing of *C. variegatus* and *O. glaber* (from the perspective of *C. variegatus*) demonstrated significantly different behavioral responses. For example, *P. megacephala* showed significantly more cases of "mandibles open" than any of the other eight behaviors. *Ochetellus glaber* showed significantly more "mutual investigation" acts than the other eight behaviors.

The group assays were analyzed by a paired t- test to assess survival within each pair. Table 3 shows the average survival in all four trials for each species within a pair. Each pair except for *C. variegatus* and *O. glaber* ($P = 0.191$) had significant differences in survival ($P < 0.05$). *Pheidole megacephala* versus *A. gracilipes* showed the highest levels of aggression, in which *P. megacephala* had 0% survival against *A. gracilipes* in all four trials. Similarly, *C. variegatus* versus *P. megacephala* showed high levels of agonism, in which *P. megacephala* suffered 0% survival in three out of the four trials. *Leptogenys falcigera* had the highest average survival rate, with 100% survival in all four trials paired with all five species. Survival of workers in control trials was high, exceeding 95%.

The most aggressive species in individual assays were *P. megacephala* and *A. gracilipes*, as they ranked first and second, respectively, in the aggression category. *Pheidole megacephala* also ranked last in the no response and defen-

Table 1. Individual agonistic assays: Total number of behavioral interactions for each ant species paired with the four other species. Nine behaviors were recorded, ranging from aggressive to defensive behavior from left to right in the table.[1,2]

Species	BT	GF	AT	MO	IN	MI	ES	BA	BB
Camponotus variegatus	8[a]	5[a]	52[ab]	62[abc]	29[ab]	123[c]	75b[c]	45[ab]	5[a]
Anoplolepis gracilipes	6[a]	9[a]	70[acd]	73[cd]	38[abc]	101[d]	71[acd]	27[ab]	3[a]
Ochetellus glaber	4[a]	51[a]	18[a]	41[a]	43[a]	108[b]	41[a]	16[a]	9[a]
Pheidole megacephala	5[a]	0[a]	21[ab]	180[c]	45[ab]	74[b]	11[a]	11[a]	0[a]
Leptogenys falcigera	1[a]	0[a]	1[a]	3[a]	157[c]	87[b]	29[a]	18[a]	2[a]

[1]BT: Bite, GF: Gaster flex, AT: Attack, MO: Mandibles open, IN: Indifference, MI: Mutual investigation, ES: Escape, BA: Being attacked, BB: Being bitten.
[2]Values in the same row followed by the same letter are not significantly different (Proc GLM, $P > 0.05$)

Table 2. Rankings of ant species in individual agonistic assays (1 is highest rank), within summary behavioral categories of aggression, no response, or defense.[1]

Species	Aggression	No response	Defensive
Camponotus variegatus	4	3	1
Anoplolepis gracilipes	2	4	2
Ochetellus glaber	3	2	3
Leptogenys falcigera	5	1	4
Pheidole megacephala	1	5	5

[1]Bite, gaster flex, attack, mandibles open included in the *aggression* category; indifference and mutual investigation are included in the *no response* category; escape, being attacked, and being bitten are included in the *defensive* category.

Table 3. Group agonistic assays: Average percent survival of ant species in each pairing. Rows show average survival when that species was paired with the species listed in each column.[1]

Species	*A. gracilipes*	*O. glaber*	*C. variegatus*	*P. megacephala*	*L. falcigera*
A. gracilipes	**100%**	85%	70%	70%	100%
O. glaber	63%	**99%**	90%	85%	100%
C. variegatus	93%	98%	**100%**	93%	100%
P. megacephala	0%	30%	8%	**98%**	100%
L. falcigera	100%	100%	100%	100%	**100%**

[1]Average survival in control trials with each species is indicated in bold font.

sive categories in comparison to the other four species. *Anoplolepis gracilipes* followed the same pattern as *P. megacephala* in the no response and defensive categories, also showing low levels of no response and defensive behavior compared to the other three species. These data are comparable to qualitative field observations at the time of collection in the spring and fall of 2005, when *P. megacephala* and *A. gracilipes* were observed to be the most common species in Waimanalo and Tantalus, respectively (Kirschenbaum & Grace 2007). The high aggression and low defensive behavior of *P. megacephala* and *A. gracilipes* parallels observation at these two collection sites and other surveys of ant distribution in Hawaii (Huddleston & Fluker 1968, Reimer *et al.* 1990, Reimer 1994). The behavioral responses of *L. falcigera* in the individual and group assays were also similar to what was observed in the field at the time of collection. *Leptogenys falcigera* was ranked first in the no response category for the individual assays and had the highest survival rate (100%) for the group assays, which would appear to be consistent with the cryptic nature of this ant in the field. Although little is known of the biology of *L. falcigera,* these studies suggest that the high levels of indifferent behavior shown in individual assays and the highest average survival in group assays may reflect the mechanism that this species uses to survive when competing against more aggressive species.

According to previous distributional surveys of ant species in Hawaii, *P. megacephala* and *A. gracilipes* tend not to overlap in their range (Fluker & Beardsley 1970, Huddleston & Fluker 1968). The other three species that were sampled (*C. variegatus, O. glaber*, and *L. falcigera*) were found in the same habitat as *P. megacephala* at Waimanalo, but not at the Tantalus location. It is possible that the higher levels of aggression of *P. megacephala* were due to exposure to the other three species in Waimanalo; and that *A. gracilipes* was not as aggressive due to lack of experience with these species. In the case of invasive ants and endemic spiders in Hawaii, Gillespie (1999) has shown how novel associations among these arthropods have led to a decrease in the native spider populations, because of lack of defense against the ants. Novel associations of *C. variegatus, O. glaber*, and *L. falcigera* with *A. gracilipes* in our laboratory assays could explain why these pairings resulted in lower levels of agonism due to lack of defensive behavior.

Based upon species abundance observed in the field, there were distinct numerically dominant and subdominant species at the time of collection. As mentioned above, previous studies have examined the community structure of numerically dominant and subdominant ant species that are commonly found in the same habitat (Morrison 1996, 2000a, Room 1975, Majer *et al.* 1994). One mechanism permitting co-occurrence of dominant and subdominant species may be temporal or spatial separation in foraging or nesting habits (Suarez *et al.* 2005). For example, *P. megacephala* is primarily a diurnal ground forager and *C. variegatus* is a nocturnal ground forager (Huddleston & Fluker 1968, Reimer *et al.* 1990). O*chetellus glaber* is an arboreal species, and *L. falcigera* is both a ground and arboreal species with infrequent occurrences and cryptic foraging strategies. These distinctions in their nesting and foraging habits may allow these four ant species to coexist in the same environment. Although these assemblages of ant species co-occur in the field, they still demonstrate aggressive behavior when forced to interact in laboratory assays.

The group assays were based on mortality as an indicator of aggression levels. The consequence of high agonism between pairs was the death of one or both species. All *P. megacephala* group trials resulted in less than 100% survival of the initial ten individuals in the trials. For example, *P. megacephala* survival varied from an average of 50% in all trials paired with *O. glaber* to total mortality of all individuals in all the trials paired against *A. gracilipes.* The results show that *P. megacephala* is indeed an aggressive species but is not necessarily successful in combat, which suggests that there are other mechanisms aside from aggression that *P. megacephala* is using in the field to become a dominant species. Large colony size is one popular theory to explain the success and numerical dominance of ant species (Walters & Mackay 2005), suggesting that perhaps more individuals would survive if larger numbers of *P. megacephala* individuals were included in the group assays. Similar agonistic assays were conducted by Fluker & Beardsley (1970) in which *P. megacephala* experienced high mortality when paired against *Linepithema humile* (Mayr) or *A. gracilipes*. Fluker & Beardsley (1970) conducted additional assays where more individuals were included in trials paired with *L. humile*, resulting in higher survival. Because *P. megacephala* was ranked first in aggression in individual assays and was highly agonistic in the group assays, the low survival of this species is perhaps at least partially due to the low number of individuals present in these assays.

The results of our individual and group assays parallel qualitative observations of abundance and numerical dominance in the field. *Pheidole megacephala* and *A. gracilipes* had the highest levels of aggression in individual assays and high agonism in group assays. Although interactions in laboratory bioassays may not fully reflect the actions of mature ant colonies under natural conditions, this reductionist approach is a useful starting point for assessing ecological interactions. As mentioned by Holway & Suarez (1999), Holway *et al.* (1998), and Morrison (2000a) in their studies of the role of behavior in successful ant invasions, agonistic assays can be useful tools for predicting which species will be numerically dominant and potentially most harmful to invaded habitats.

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