Agonistic Responses of the Tramp Ants *Anoplolepis gracilipes, Pheidole megacephala, Linepithema humile,* and *Wasmannia auropunctata* (Hymenoptera: Formicidae)

by

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ABSTRACT

Using both individual and group behavioral assays, we evaluated agonistic interactions among four introduced ant species in Hawaii: *Anoplolepis gracilipes* (Smith), *Pheidole megacephala* (Fabricius), *Linepithema humile* (Mayr), and *Wasmannia auropunctata* (Roger). Overall results from the individual assays indicated a high degree of agonism between *P. megacephala* and *A. gracilipes*, and between *P. megacephala* and *W. auropunctata*. *Anoplolepis gracilipes* consistently had the highest average survival in group assays. *Pheidole megacephala* had the lowest average survival in trials without soldiers present; but when *P. megacephala* soldiers were present, *L. humile* had the lowest average survival. *Pheidole megacephala* and *L. humile* demonstrated the most defensive behavior when paired individually with *W. auropunctata*, as well as suffering high mortality in group assays when paired against *W. auropunctata*. Although *A. gracilipes* has the highest average survival in group assays, this species is restricted to particular nesting sites in the field and therefore limited in its potential for expansion. In conjunction with previous studies, our results suggest *W. auropunctata* has the potential to become a behaviorally and numerically dominant ant species in Hawaii.

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

INTRODUCTION

The Hawaiian archipelago has a relatively large proportion of endemic arthropods and plants, but the ca. 45 ant species present in Hawaii are all

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introduced (Wilson & Taylor 1967, Reimer *et al.* 1990). Researchers in invasive ant biology have proposed a number of theories to explain species dominance, including heightened interspecific aggression, lessened intraspecific aggression, recruitment efficiency, and large colony size (Wilson 1971, Holldobler & Wilson 1990, Holway *et al.* 2002, Walters & Mackay 2005). The term "tramp ant" (Passera 1994) describes species that are consistent with the above mentioned theories and thus very likely to become established in new habitats.

Unlike previous agonistic assays we conducted using a dominant and subdominant invasive species to assess competitive mechanisms that influence community structure in Hawaii (Kirschenbaum & Grace 2007), this study addressed behavioral interactions among exclusively numerically dominant ant species (Kirschenbaum & Grace 2008). As in the previous work, we tested agonism both between pairs of individual ants, and between groups. The ant species we selected, *Anoplolepis gracilipes* (Smith), *Pheidole megacephala* (Fabricius), *Linepithema humile* (Mayr), and *Wasmannia auropunctata* (Roger) are considered to be among the most invasive and destructive species in Hawaii and in the world (Holway *et al.* 2002, Reimer 1994, Krushelnycky *et al.* 2005.

These four ant species are found on the major Hawaiian Islands and are listed in "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Group (ISSG 2006). *Anoplolepis gracilipes*, the long-legged ant, is speculated to originate from West Africa, India or China. This species has a widespread distribution on all the major Hawaiian Islands and is restricted to rocky nesting sites (Zimmerman 1953, Fluker & Beardsley 1970). *Pheidole megacephala* (big-headed ant) was first discovered in Hawaii in 1879 (Smith 1879) and prefers areas with light rainfall and elevations below 600m (Fluker & Beardsley 1970). ***Linepithema humile***, the Argentine ant, originally from South America also prefers light rainfall, has adapted to higher elevations in Hawaii of 900m-1800m and was first discovered in Hawaii in 1940 (Fluker & Beardsley 1970, Zimmerman 1941). *Wasmannia auropunctata*, the little fire ant, is originally from South America, is the most recent invader to Hawaii (1999) and occurs on the Island of Hawaii and in a single locality on Kauai (Conant & Hirayama 2000).

MATERIALS AND METHODS

Collection of Ants

We collected *L. humile* from Puu Huluhulu on the island of Hawaii. This collection site is in a national park protecting a "kipuka," a raised vegetated area surrounded by a younger lava flow. *Wasmannia auropunctata* was collected from Papaiko, a small town outside of Hilo on the island of Hawaii. The site was on a small fruit orchard with mangosteen and rambuttan trees. *Pheidole megacephala* was collected from the Waimanalo Experiment Station of the University of Hawaii, on the windward side of the island of Oahu. *Anoplolepis gracilipes* was collected in the Tantalus Forest Reserve above Honolulu, Hawaii, on the opposite side of the Koolau mountain range from the Waimanalo Station. Individuals and groups used in the bioassays were drawn from several different colonies of each species.

Individual Bioassays

Interactions between individuals of each species were evaluated using a rating system with a range of nine behaviors. 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 minutes to acclimate before another individual from either the same or one of the other three ant species was likewise transferred to the opposite end of the dish. We recorded any behavioral interactions occurring during the next ten minutes using the methods of Retana and Cerda (1995), with the 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. Each response was noted and totaled, with five replicates for each species pairing. Only the minor castes were used for species with polymorphic colony structures. Behavioral interactions were recorded from the perspective of each of the ant species in the trial, so that both primary aggression and stimulated aggression in response to attack (i.e. biting and being bitten) were accounted for in each species.

Group Bioassays

Group agonistic assays consisted of ten individuals from each species paired in observation arenas. In addition to using minor castes, as in the individual assays, group assays were also conducted that included 10% *P. megacephala* soldiers in the groups. This may more realistically simulate field conditions with this species.

Group assays were evaluated on the basis of ant mortality, under the assumption that aggressive interactions lead to mortality. Ten individuals from each species were placed in 400 ml glass containers coated with fluon around the edges. Paired species were left for three hours, with four replications of each species pairing. Controls (same species pairings) 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. The number of living individuals of each species was recorded to assess survival.

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 the GLM Procedure in SAS. T-tests were used to test mean difference in survival among pairs in group assays (SAS Institute, 2002-2003). Means were separated with the Tukey's Studentized Range (HSD) Test to determine significant behavioral responses in the individual assays.

RESULTS

Individual assays

Outcomes for total possible behaviors for each of the species interactions are illustrated in Table 1, showing significant differences (HSD procedure) among the behaviors for each species. *Pheidole megacephala* showed the highest frequency of aggressive behavior with 89 acts of 'mandibles open' and 23 acts of 'attack,' more than double that of the other species. Table 3 summarizes the nine behaviors into three categories of aggression, no response, and defensive response, and indicates the relative rank of each species within these categories. By chi-square analysis, each species could be placed into one of the three categories. *Pheidole megacephala* ranked first in the aggression category and elicited significantly greater frequencies of aggressive behavior ($\chi^2 = 49$, df = 2, $P < 0.001$). *Linepithema humile* ranked second in both the aggression and defensive categories, but did not show any particular pattern of behavior towards any of the other species (Table 2-3). *Pheidole megacephala* did, however, show the highest aggressive behavior towards *A. gracilipes* and

Table 1. Individual agonistic assays: Total behavioral interactions for each species when paired with the three 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
Anoplolepis gracilipes	0[a]	13[a]	4[a]	18[a]	57[b]	68[b]	47[b]	9[a]	2[a]
Pheidole megacephala	6[ab]	0[ab]	23[a]	89[b]	12[ab]	58[c]	3[ab]	12[ab]	11[ab]
Linepithema humile	0[a]	7[a]	11[ab]	29[bc]	6[a]	46[c]	7[a]	10[ab]	2[a]
Wasmannia auropunctata	16[a]	0[a]	7[a]	14[a]	61[b]	50[b]	0[a]	7[a]	7[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 > 0.050).

Table 2. Summary behavioral interactions between each pair of ant species in individual assays.[1]

P. megacephala response to other species		
W, auropunctata	*L. humile*	*A. gracilipes*
Aggressive-44	Aggressive-15	Aggressive-59
No response-28	No response-20	No response-22
Defenensive-20	Defenensive-5	Defensive-1
L. humile **response to other species**		
W. auropunctata	*P. megacephala*	*A. gracilipes*
Aggressive-2	Aggressive-21	Aggressive-24
No response-13	No response-19	No response-20
Defensive-10	Defensive-6	Defensive-3
W. auropunctata **response to other species**		
L. humile	*P. megacephala*	*A. gracilipes*
Aggressive-13	Aggressive-31	Aggressive-3
No response-11	No response-44	No response-56
Defensive-0	Defensive-14	Defensive-0
A. gracilipes **response to other species**		
L. humile	*W. auropunctata*	*P. megacephala*
Aggressive-10	Aggressive-14	Aggressive-11
No response-23	No response-62	No response-40
Defensive-19	Defensive-6	Defensive-33

[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.

second highest towards *W. auropunctata* (Table 2). Likewise, *W. auropunctata* showed the highest number of aggressive acts towards *P. megacephala*, and *A. gracilipes* showed the highest number of defensive acts towards *P. megacephala*. These results indicate a high degree of agonism between *P.*

Table 3. 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
Anoplolepis gracilipes	4	2	1
Pheidole megacephala	1	4	3
Linepithema humile	2	3	2
Wasmannia auropunctata	3	1	4

[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 4. 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*	*P. megacephala*	*L. humile*	*W. auropunctata*
A. gracilipes	100%	78%	75%	78%
P. megacephala (soldiers absent)	0%	95%	33%	0%
P. megacephala (soldiers present)	55%	95%	15%	38%
L. humile	18%	85%	95%	3%
W. auropunctata	43%	65 %	38%	95%

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

megacephala and *W. auropunctata*, and between *P. megacephala* and *A. gracilipes* in individual assays. *Anoplolepis gracilipes* showed the highest number of 'indifference', 'mutual investigation', and 'escape' behaviors throughout all pairings (P < 0.001), ranking it first in the defensive category and second in the no-response category (Table 3). *Anoplolepis gracilipes* showed its highest number of non-responsive acts towards *W. auropunctata,* and similarly, *W. auropunctata* showed the highest number of non-responsive acts towards *A. gracilipes*. The low levels of agonism between *W. auropunctata* and *A. gracilipes* in the individual assays suggest that these two species do not recognize each other as competitors.

Group assays

In assays without soldiers present, *P. megacephala* had the lowest survival, with an average of 11% survival for all trials (Table 4). When paired with *A. gracilipes* and *W. auropunctata*, *P. megcephala* had 0 % survival in all four replications. *Linepithema humile* had the second lowest average survival at 33% with the lowest highest average survival at 3% when paired with

W. auropunctata. *Wasmannia auropunctata* resulted in an average of 49% survival in all pairings, with lowest survival when paired against *L. humile* at 38%. *Anoplolepis gracilipes* had the highest survival, with an average 77% in all trials. All species paired with *P. megacephala* without soldiers present exhibited a significant difference in mean survival for each pairing ($P < 0.050$). *A. gracilipes* and *L. humile* also evidenced a significant difference in mean survival ($P < 0.050$).

The trials that included *P. megacephala* soldiers resulted in approximately the same average survival for *A. gracilipes* and *W. auropunctata*, but drastically changed the average survival for *P. megacephala* and *L. humile* (Table 4). When soldiers were present, *P. megacephala* had the second lowest average survival (higher than when soldiers were not present) and *L. humile* suffered the lowest average survival. *Anoplolepis gracilipes* consistently had the highest survival with an average of 83% and 77% in trials with and without *P. megacephala* soldiers respectively. *Wasmannia auropunctata* also showed a relatively constant average survival of 42% to 49% with or without *P. megacephala* soldiers, respectively. There were no significant differences in mean survival between any pairs when *P. megacephala* soldiers were present.

DISCUSSION

Our results from both the individual and groups assays are similar to those of Fluker & Beardsley (1970), who studied agonistic interactions among *P. megacephala, L. humile* and *A. gracilipes*. Throughout all the assays we conducted, *P. megacephala* was the most aggressive species and consistently initiated attack in all paired trials. In agreement with the results of Fluker & Beardsley (1970), this species was extremely aggressive, but was not entirely successful in killing its opponents. When *P. megacephala* soldiers were added to group trials, this species became more successful in attack, reducing the average survival rate of *W. auropunctata* and *L. humile*. Clearly, soldiers assist *P. megacephala* in combat against the other two species, although *P. megacephala* mortality was still relatively high at an average at 64%. Fluker & Beardsley (1970) showed that an increase in the number of *P. megacephala* individuals included in agonistic trials did indeed result in increased success against *L. humile*. Thus, aggressive behavior coupled with large colony size appear to contribute to the success of *P. megacephala* in the field.

Linepithema humile ranked second in the defensive and aggression categories, demonstrating high agonism in individual trials. However, in group trials *L. humile* exhibited the lowest average survival among all species when *P. megacephala* soldiers were present. In these laboratory assays, *Linepithema humile* behaved similarly to *P. megacephala* in that it is an aggressive species, but not very successful in group combat. The major difference between the behavior of *L. humile* and *P. megacephala* is that *L. humile* is less inclined to initiate attack, but when provoked it will defend itself (Fluker & Beardsley 1970). In previous studies with *L. humile*, it has been shown to owe much of its invasive success to its large colony size (Walters & Mackay 2005). This may be the driving mechanism that allows this species to be dominant in the field, while its aggressive behavior is only complementary to its success.

Pheidole megacephala and *L. humile* were the most aggressive species in individual assays, both exhibiting their most aggressive behavior towards *A. gracilipes*. Not only was *A. gracilipes* a threat to *P. megacephala* and *L. humile* in individual assays, this species consistently had the highest average survival in the group assays. Fluker & Beardsley (1970) also observed that *A. gracilipes* was successful in group combat, and related this success to its toxic defensive sprays. Spraying was also observed in our study, with 13 counts of gaster flex, the highest of all the species. The invasive success of this species as been observed in research on Christmas Island and Bird Island, where *A. gracilipes* abundance and dominance have been found to have negative effects on native vertebrate populations (Abbott 2005). Although highly agonistic, *A. gracilipes* is limited in its expansion in the Hawaiian Islands due to the lack of resources, e.g. rocky nesting sites, and competition from other dominant species (Wilson & Taylor 1967, Fluker & Beardsley 1970).

Pheidole megacephala and *L. humile* showed their most defensive behavior when paired against *W. auropunctata,* suggesting that this species may be a threat to both *P. megacephala* and *L. humile*. *Wasmannia auropunctata* on the other hand was the least threatened by *A. gracilipes,* showing the highest frequency of non-responsive acts in individual paired trials. Although *W. auropunctata* did not rank first in the aggression category, this species still exhibited high levels of agonism in paired trails with *P. megacephala* and *L. humile*. Furthermore, this species had the highest overall frequency of biting behavior compared to all other species, as well as highest agonistic behavior from *P. megcephala* and *L. humile* in the individual paired trials. Based on our results, it can be concluded that this new invader is a competitive threat to *P. megacephala* and *L. humile,* and will influence the distribution of these two species.

When the results of our assays are considered in conjunction with the results of previous studies, we can predict that *W. auropunctata* has great potential to become Hawaii's next numerically and behaviorally dominant ant species. Although *P. megacephala* and *L. humile* were more aggressive in individual assays, they exhibited their most defensive behavior when paired with *W. auropunctata*, as well as suffering high mortality in group assays when paired against *W. auropunctata*. *Anoplolepis gracilipes* had the highest average survival in group assays, but this species is restricted to particular nesting sites in the field that likely limit its expansion. In other regions, *W. auropunctata* has been a very destructive invader (Le Breton *et al*. 2003, 2004, 2005; Wetterer & Porter 2003).

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