# Agonistic Interactions of Four Ant Species Occurring in Hawaii with *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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## ABSTRACT

Of the ca. 44 ant species known to occur in Hawaii, there has been limited research on *Leptogenys falcigera* Roger, *Camponotus variegatus* Smith, *Anoplolepis gracilipes* Smith, and *Wasmannia auropunctata* Roger; all of which were recorded as adventive species in Hawaii as early as the late 1800s to as recently as 1999. In laboratory assays, we assessed agonistic interactions of these ant species with the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, another extremely invasive social insect. These assays provide information on ecological interactions and the invasive biology of these four ants. We used a rating system based on the first two behavioral responses of the two insects paired in each bioassay. Following published methods, the rating system was as follows: avoidance (-1), no response (0), and aggression (+1). Of the four ant species used in this study, *W. auropunctata* demonstrated greater aggression towards *C. formosanus* than the other ants. *Coptotermes formosanus*, in turn, showed less aggressive behavior towards *W. auropunctata* than towards *L. falcigera* and *C. variegatus*. *Wasmannia auropunctata* is the most recently introduced ant species in Hawaii, and may demonstrate more interspecific aggression, but may not be recognized by or elicit an aggressive response from *C. formosanus*.

## INTRODUCTION

Of the 44 ant species known to occur in Hawaii (Wilson & Taylor 1967, Reimer *et al*. 1990, Krushelnycky *et al*. 2005), there has been limited research on the invasive species *Leptogenys falcigera* Roger (sickle-tooth ant), *Camponotus variegatus* Smith (Hawaiian carpenter ant), *Anoplolepis gracilipes*

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Smith (long-legged ant), and *Wasmannia auropunctata* Roger (little fire ant); and none on their interactions with the subterranean termite *Coptotermes formosanus* Shiraki. All of the ant species found in the Hawaiian Islands are invasive (Wilson & Taylor 1967, Reimer 1994), which creates a unique system in which to study invasion biology. Several reports have shown that ants have devastating impacts on native flora and fauna (Holway 1998, Holway *et al*. 2002, Le Breton *et al*. 2004, Krushelnycky *et al*. 2005, Reimer 1994). Many factors contribute to ants being successful colonizers including low intraspecific aggression (unicolonial nests), high interspecific aggression, and mutualistic relationships with Hemipteran insects (Holway *et al*. 2002, O'Dowd *et al*. 2003, Helms & Vinson 2003). Our study focused on competitive characteristics that may lead to the success of invasive ant species in Hawaii.

We selected both dominant and subdominant ant species for this study. Dominance may reflect the extent of time since initial introduction to Hawaii. *Leptogenys falcigera* is an old world species and one of the original ants to colonize the Hawaiian Islands in the late 1800's (Smith 1879). This nomadic ant from the Ponerini tribe forms small colonies (50-100 workers) and has ergatoid (worker-like) queens. It feeds mainly on isopods and inhabits dry soil and tree cavities. *Camponotus variegatus* is native to Southeastern Asia and is also documented as one of the pioneering ant species to arrive in the Hawaiian Islands (Smith 1879). *Camponotus variegatus* also produces smaller colonies with approximately 100 workers, soldiers and one queen. This ant will feed on sugar-based foods as well as dead insects. *Anoplolepis gracilipes* is speculated to have originated from West Africa, India or China (Wetterer 2005). This species has a widespread distribution on all the major Hawaiian Islands. *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). *Wasmannia auropunctata*, originally from South America, is the most recent invader to Hawaii and has been recorded on the Island of Hawaii and in a single locality on Kauai. *Wasmannia auropunctata* is a known tramp ant species and is also listed by ISSG (2006). Originally from China, the Formosan subterranean termite, *C. formosanus,* is Hawaii's most economically damaging insect pest (Yates & Tamashiro 1990) and currently occurs throughout the Hawaiian Islands.

*Leptogeny. falcigera* and *C. variegatus* were first recorded in Hawaii in the late 1800s, and *A. gracilipes* and *W. auropunctata* were discovered more recently in 1952 and 1999 respectively (Krushelnycky *et al.* 2005, Conant & Hirayama 2000, Smith 1879, Zimmerman 1953). Perhaps the dominance of *A. gracilipes* and *W. auropunctata* is related to their more recent introduction into Hawaii, and an ability to outcompete *L. falcigera* and *C. variegatus*. As ants are major predators of termites (Deligne *et al*. 1981), we conducted experiments to investigate potential agonistic interactions among these species. Thus, in a limited fashion, we tested the hypothesis that recently arrived ant species demonstrated higher aggressive behavior than long-established species.

## METHODS

### Termite and ant collections.

Formosan subterranean termites, *C. formosanus*, were collected on the Manoa Campus of the University of Hawaii using a trapping technique designed by Tamashiro *et al.* (1973). The termites are lured to a wooden stake placed about ten centimeters below grade, which is then enclosed by a wooden trap, therefore making the termites easily assessable for collection. Termites were sampled from three colonies on the campus. In order to minimize stress, termites were extracted from the traps and used the same day for experimentation.

The ant species *L. falcigera* and *C. variegatus* were collected from the soil or within the branches or trunks of trees at an experimental farm in Waimanalo, Oahu, Hawaii. *Anoplolepis gracilipes* was collected from Tantalus Forest Reserve above Honolulu in Hawaii. The ants were carefully extracted from their original nests and reared in the laboratory at ca. 25°C. They were fed on a combination of 25% aqueous sucrose solution, peanut butter, sweet corn, tuna fish in oil, and dead insects. *Leptogenys falcigera* main diet was 25% sucrose solution and isopods. *Wasmannia auropunctata* was collected on the Island of Hawaii in the Puna district from the soil of a flower pot, and all foraging ants were assumed to come from the same colony. Since this species could not be moved between islands due to quarantine restrictions, agonistic assays were conducted on the Island of Hawaii with termites brought from the Manoa campus.

## Experimental design.

Agonistic bioassays between the ants and termites were conducted in a 60 x 15mm Petri dish, following the methods of Cornelius and Grace (1994). A termite soldier was gently placed in a Petri dish with the sides coated with fluon. Once the termite began to move around the Petri dish a worker from one of the four ant species was added to the arena. Responses were observed and recorded for both the ants and the termites as follows: avoidance (-1), no response (0), or aggression (1). Avoidance behavior was recorded if the termite or ant made physical contact and then immediately went in the opposite direction. No response was scored when no other visible behavior occurred after physical contact between the termite and ant. Aggressive behavior included biting and open mandibles. The first two interactions were observed in each assay, and the highest response was recorded. In a blocking design, individuals from each of three ant colonies of each species were paired with soldiers from each of three termite colonies, with 20 replications of each pairing.

## Statistical analyses.

To compare responses both among species, and among pairings within each species, percent mean responses of the ants and termites were transformed by the arcsine of the square root, and subjected to general linear models analysis using Proc GLM (SAS 9.1). Means were separated with the Ryan- Einot-Gabriel-Welsch multiple Range Test.

# RESULTS

The results of the overall behavioral analysis of the four ant species showed that *W. auropunctata* demonstrated significantly more "no response" ($p=0.0146$, Table 1) and "aggressive" ($p= 0.0053$, Table 1) behavior towards *C. formosanus* than the other ant species. There were no significant differences in the overall responses of *L. falcigera*, *C. variegatus,* and *A. gracilipes* towards *C. formosanus* (Table 1). *Coptotermes formosanus* showed the lowest overall aggressive response towards *W. auropunctata,* in comparison to responses to *L. falcigera* and *C. variegatus* ($p= 0.0410$, Table 2).

The trials with *L. falcigera, A. gracilipes*, and *C. variegatus* showed that "no response" was the most frequently observed behavior of the ants towards *C. formosanus* ($p< 0.0001$, Table 3). *Coptotermes formosanus* elicited a signifi-

Table 1. Mean percentage behavioral response of four ant species to *C. formosanus* soldiers.

| | ANT RESPONSE | | |
|---|---|---|---|
| | Avoid | No response | Aggressive |
| *L. falcigera* | 11 ± 0.04[a] | 87 ± 0.06[a] | 2 ± 0.02[a] |
| *C. variegatus* | 2 ± 0.02[a] | 90 ± 0.06[a] | 8 ± 0.04[a] |
| *A. gracilipes* | 5 ± 0.03[a] | 82 ± 0.11[a] | 13 ± 0.09[a] |
| *W. auropunctata* | 3 ± 0.02[a] | 38 ± 0.06[b] | 59 ± 0.04[b] |

*Percentages in the same column followed by the same letter are not significantly different (p>0.05, Proc GLM).

Table 2. Mean percentage behavioral response of *C. formosanus* soldiers to four ant species.

| | TERMITE RESPONSE | | |
|---|---|---|---|
| | Avoid | No response | Aggressive |
| *L. falcigera* | 0 ± 0.00[a] | 27 ± 0.14[a] | 73 ± 0.14[a] |
| *C. variegatus* | 0 ± 0.00[a] | 23 ± 0.16[a] | 77 ± 0.16[a] |
| *A. gracilipes* | 0 ± 0.00[a] | 48 ± 0.04[a] | 52 ± 0.04[ab] |
| *W. auropunctata* | 8 ± 0.06[a] | 73 ± 0.04[a] | 19 ± 0.07[b] |

*Percentages in the same column followed by the same letter are not significantly different (p>0.05, Proc GLM).

cantly greater frequency of "aggression" behavior towards both *L. falcigera* and *C. variegatus* (respectively; p= 0.0118, p=0.0173, Table 3). In the case of *C. formosanus* and *A. gracilipes,* the termites were observed to "avoid" more frequently in the trials (p= 0.0011, Table 3). In the case of *W. auropunctata* and *C. formosanus*, *W. auropunctata* was observed to have the highest aggressive response towards *C. formosanus* (p= 0.0003, Table 3) and *C. formosanus* showed a higher "no response" behavior towards *W. auropunctata* (p= 0.005, Table 3).

## DISCUSSION

Previous studies conducted by Cornelius and Grace (1994, 1995) on the agonistic interactions of *C. formosanus* and ants demonstrated that the termites showed more frequent aggressive behavior towards the ants than vice-versa. The aggression elicited in the Cornelius and Grace (1994, 1995) assays is suggested to be a defensive trait of termite soldiers evolved in response to

Table 3. Behavioral response within each ant-termite pair, from the perspective of both the ant and the termite.

| ANT RESPONSE | | TERMITE RESPONSE | |
|---|---|---|---|
| Behavior | Percent mean | Behavior | Percent mean |
| 1) *L. falcigera* vs. *C. formosanus* | | 1) *C. formosanus* vs. *L. falcigera* | |
| Avoid | 11 ± 4.41a | Avoid | 0 ± 0 a |
| No response | 87 ± 6.01b | No response | 27 ± 14.2 a |
| Aggression | 2 ±1.67a | Aggression | 73 ± 14.2 b |
| 2) *C. variegatus* vs. *C formosanus* | | 2) *C. formosanus* vs. *C.variegatus* | |
| Avoid | 2 ± 1.67 a | Avoid | 0 ± 0 a |
| No response | 90 ± 5.77 b | No response | 23 ± 16.4 a |
| Aggression | 8 ± 4.41 a | Aggression | 77 ± 16.4 b |
| 3) *A. gracilipes* vs. *C. formosanus* | | 3)*C. formosanus* vs. *A. gracilipes* | |
| Avoid | 5 ± 2.89 a | Avoid | 0 ± 0 a |
| No response | 82 ± 10.9 b | No response | 48 ± 4.41 b |
| Aggression | 13 ± 8.82 a | Aggression | 52 ± 4.41 b |
| 4) *W. aurounctata* vs. *C. formosanus* | | 4) *C. formosanus* vs. *W. auropunctata* | |
| Avoid | 33 ± 30.9 a | Avoid | 8 ± 6.01 a |
| No respons | 38 ± 6.01 b | No respons | 73 ± 4.41 b |
| Aggression | 59 ± 4.41 c | Aggression | 18 ± 7.26 a |

*Percentages in the same column followed by the same letter are not significantly different ($p>0.05$, Proc GLM).

predation by ants (Deligne *et al*. 1981). Our analysis of the interactions of *L. falcigera*, *C. variegatus* and *A. gracilipes* with *C. formosanus* gave a pattern that is consistent with these previous agonistic studies.

The termite behavior that was observed most frequently was aggression (i.e., open mandibles, biting and attacking). These aggressive responses by *C. formosanus* were elicited by all of the ant species except *W. auropunctata*, to which *C. formosanus* showed the highest level of non-responsive behavior. The behavior shown by *L. falcigera*, *C. variegatus*, and *A. gracilipes* towards *C. formosanus* was non- responsive the majority of the time. *Wasmannia auropunctata* was unique in showing much higher levels of aggression towards *C. formosanus* (Table 1).

Studies of invasion biology have found generally high interspecific aggression among introduced tramp ants towards other ants and arthropod species (Passera 1994, Holway & Suarez 1999, Morrison 2000) In our laboratory assays, this high level of aggression was certainly observed in *W. auropunctata*,

the most recent invader to Hawaii. Tramp ants are characterized as having decreased intraspecific aggression and increased interspecific aggression; and they often form large multi-colonial nests (Holway 1998, Suarez *et al.* 1999, Holway *et al*. 2002, Tsutsui & Suarez, 2003). Tsutsui *et al*. (2000, 2003) suggests that this decreased intraspecific aggression and increased interspecific aggression in introduced species is due to a reduction in genetic variability between colonies, resulting in a propensity to recognize individuals from different colonies as nestmates. This phenomenon has been explored in recent studies by Le Breton *et al*. (2004) that show *W. auropunctata* having higher interspecific aggression and lower intraspecific aggression in new habitats than in its native range.

Although *A. gracilipes* is noted to have polygynous colonies in introduced habitats (Abbott 2005), in our assays this species did not show the same levels of aggression as *W. auropunctata*. Perhaps the difference in behavior of these two species may be attributable to the time of introduction into Hawaii. The aggressive behavior towards *C. formosanus* could be due to the lack of experience of *W. auropunctata* with *C. formosanus,* whereas the other ant species may have stabilized their behavior over time to show less aggression towards this essentially non-threatening termite. In reviewing publications on the ecology, policy and management of ants in Hawaii, Krushelnycky *et al*. (2005) proposed that the current ant assemblage is approaching an equilibrium, with the exception of turnover in certain dominant species. *Anoplolepis gracilipes* and *W. auropunctata* are both considered to be highly invasive species (ISSG 2006), and *W. auropunctata* may thus be replacing *A. gracilipes* in the dominance hierarchy. Additional behavioral assays, including a wider range of behaviors than were included in the present study may be useful to further illuminate the invasion biology of Hawaii's ant fauna.

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