Inter- and Intraspecific Aggression in the Invasive Longlegged Ant (Hymenoptera: Formicidae)

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ABSTRACT The longlegged ant, *Anoplolepis gracilipes* (Fr. Smith) (Hymenoptera: Formicidae), is a highly invasive species that can aggressively displace other ant species. We conducted laboratory assays to examine interspecies aggression of *A. gracilipes* versus 15 sympatric ant species found in the urban environment and disturbed habitat in Malaysia: *Monomorium pharaonis* (L.), *Monomorium floricola* (Jerdon), *Monomorium orientale* Mayr, *Monomorium destructor* (Jerdon), *Pheidole parva* Mayr, *Crematogaster* sp., *Solenopsis geminata* (F.), *Tapinoma indicum* (Forel), *Tapinoma melanocephalum* (F.), *Technomyrmex butteli* Forel, *Dolichoderus thoracicus* (Smith), *Paratrechina longicornis* (Latrielle), *Oecophylla smaragdina* (F), *Camponotus* sp., and *Tetraponera rufonigra* (Jerdon). *A. gracilipes* showed aggressive behavior toward all opponent species, except the smallest *M. orientale*. Opponent species size (body size, head width, and mandible width) was significantly correlated with *A. gracilipes* aggression level and mortality rate. We also found a significant positive relationship between *A. gracilipes* aggression level and the mortality of the opponent species. The results suggest that invasive populations of *A. gracilipes* would have the greatest impact on larger ant species. In addition, we examined the intraspecific aggression of *A. gracilipes*. We found that *A. gracilipes* from different localities in Malaysia showed intraspecific aggression toward one another. This finding differs from the results of studies conducted in Christmas Island earlier. Differences in the genetic variability among populations may explain these differing results.

KEY WORDS *Anoplolepis gracilipes*, interspecific, intraspecific, aggression, invasive species

Recognition of nestmates and discrimination of intruders are important behavioral aspects in eusocial insects that help to maintain the stability of insect societies (Hölldobler and Wilson 1990). According to Howse (1983), the most common and pernicious enemies of social insects are the other social insects, especially the carnivorous ant species. They compete for food, space, and other resources, and this competition induces aggressive or agonistic behaviors (Thorne and Haverty 1991). Hölldobler and Wilson (1990) reported that the most serious competitors in ant communities are dominant territorial species, particularly those of other species.

Anoplolepis gracilipes (Fr. Smith) (Hymenoptera: Formicidae) is notoriously known as one of the 100 world most invasive species (ISSG 2007). The impact of this species includes decimation of endemic species, changes in habitat structure and resource availability, loss of biodiversity, alterations in ecosystem processes, and economic damage to livestock (Haines et al. 1994, O'Dowd et al. 2003, Lester and Tavite 2004, Abbott 2006). On Christmas Island, it was estimated that 15–20 million native red land crabs have been killed by *A. gracilipes* because supercolonies of this species were first noted there in 1989. *A. gracilipes* is also an agricultural pest, tending sap-sucking scale insects on crops. They also attacked and killed the newly hatched chickens and newly born domestic animals such as cats, dogs, pigs, and rabbits, whereas older animals were irritated (Haines and Haines 1978). Lester and Tavite (2004) reported that a significant reduction in ant species diversity has occurred with increasing *A. gracilipes* density in newly invaded areas in Tokelau Islands in South Pacific.

In the Seychelles, *A. gracilipes* is a nuisance to humans and interfering with farm workers (Haines et al. 1994). It is a severe household pest and a nuisance in public buildings, hotels, hospitals, and food and drink processing establishments in Seychelles (Lewis et al. 1976). This species has successfully spread throughout the world through a variety of pathways, such as with sea cargo involving that timber trading; in soil, machinery, and road vehicles; and within horticulture and material packaging (Chong and Lee 2009). At present, it is also one of the most important structure-invading ant species to the pest management industry in Southeast Asia (Lee and Tan 2004, Lee 2007).

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Over the past several years, we observed increasing infestations of *A. gracilipes* in the urban areas and other disturbed environments in Malaysia. To better understand the interaction between different populations of *A. gracilipes,* and its interactions with other sympatric structure-invading ant species in Malaysia, we examined the inter- and intraspecific aggression of *A. gracilipes.* The outcome of these tests may be useful to predict the species dominance of *A. gracilipes* and its potential ecological effects it may have on the other sympatric ant species.

Materials and Methods

Insects. *A. gracilipes, Monomorium pharaonis* (L.), *Monomorium floricola* (Jerdon), *Monomorium orientale* Mayr, *Pheidole parva* Mayr, *Crematogaster* sp., *Tapinoma indicum* (Forel), *Tapinoma melanocephalum* (F.), and *Technomyrmex butteli* Forel were obtained from the stock culture of the Urban Entomology Laboratory, Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia. *Monomorium destructor* (Jerdon), *Solenopsis geminata* (F.), *Dolichoderus thoracicus* (Smith), *Paratrechina longicornis* (Latrielle), *Oecophylla smaragdina* (F.), *Camponotus* sp., and *Tetraponera rufonigra* (Jerdon) were collected from the field at the Minden Campus, Universiti Sains Malaysia, Penang Island, Malaysia.

Three field populations of *A. gracilipes* used in this study, were originally collected from 1) Bukit Mertajam (BM), Penang, Malaysia; 2) Minden Campus (MC), Universiti Sains Malaysia, Penang Island, Malaysia; and 3) Matang (MTG), Sarawak, Malaysia. BM, located over the mainland of Peninsular Malaysia), is separated from MC by the Straits of Penang. The distance between MC and BM is 26 km. MTG, located in Sarawak in Borneo Island, is separated from Peninsular Malaysia (where BM and MC are located) by the South China Sea. MTG is 1,187.2 and 1,161.8 km away from MC and BM, respectively.

Morphological Measurements. The body size (from the tip of the mandible to the tip of the gaster), head width, and mandible width of the studied worker ants were measured under an SZ61 stereomicroscope (Olympus, Tokyo, Japan) connected to a computer by using analySIS Image Processing software (Soft Imaging System GmbH, Münster, Germany).

Interspecific Aggression. Tests were conducted using *A. gracilipes* and the 15 ant species mentioned above. Two assays were carried out: individual and group assays.

Individual Assay. Confrontation between individual ants was conducted by introducing a single forager worker of *A. gracilipes* with a single forager of the opponent species (Retana and Cerdá 1995, Abbott 2005, Kirschenbaum and Grace 2008a). These individuals were provided with 10% sucrose solution and a moist cotton bung and kept in groups of 10 for 12 h before the assay. A population of *A. gracilipes* (MC) was tested against each opponent species. The test was replicated 10 times for each population. The ants were placed together in a 50-ml vial (40 mm in diameter) with its inner wall coated with a thin layer of fluon. Immediately after they were introducing into the test vial, stopwatch was started and the interaction between the ants was observed for the entire 15 min and scored according to the following six (numbered 0–5) aggression behavioral indices (modified after Carlin and Hölldobler 1986, Abbott 2005): 0, casual tolerance; huddling together; allogrooming; exchanging food; 1, initial jerking back then tolerance; initial or weak avoidance; weak open-mandible threat; 2, intense antennation; jerking back at each encounter; strong open-mandible threat; 3, strong avoidance or flight; lift up of abdomen for chemical defense; 4, repeated, rapid forward and backward jerking with open mandibles; carrying the intruder; and 5, seizing or dragging legs and antennae; biting; chasing; gaster twisted forward to spray formic acid; sting; locking together; sparring.

Each of the six behaviors exhibited by *A. gracilipes* against each opponent species was recorded for its frequency of occurrence. The outcome of the interaction (win, lose, both survive, both dead) also was noted where win represents *A. gracilipes* survived the test (whereas the opponent species was killed) and lose represents *A. gracilipes* was killed (whereas the opponent species survived).

Group Assay. Ten workers each of *A. gracilipes* and the opponent species were placed together in a polyethylene container (16 by 10 by 6.5 cm) as test arena with its inner wall lined with a thin layer of floun. These individuals were provided with 10% sucrose solution and a moist cotton bung and kept in groups of 10 for 12 h before the assay. One population of *A. gracilipes* (MC) was used for the assay. For species with dimorphic workers, both sizes were separately tested. Mortality of each tested species was recorded at 5, 10, 20, 30, and 60 min; then hourly up to 8 h; and finally 24 h postintroduction. After 8 h, the ants were provided 10% sucrose solution and a moist cotton bung. The test was replicated five times for each population of *A. gracilipes.* No mortality was found in the control set during the entire evaluation period, indicating that mortality only occurred due to the effects of aggression. The mean mortality of all replicates was used to determine the level of susceptibility for both *A. gracilipes* and the opponent species, where 0% is not susceptible (NS), >0–<25% is less susceptible (LS), 25–<50% is moderately susceptible (MS), 50–<75% is susceptible (S), and 75–100% is very susceptible (VS).

Data Analysis. The relationship between the mean frequency of behavioral index 5 of *A. gracilipes* and mean body size of the opponent species was determined with simple linear regression, whereas the relationship between mean percentage of mortality of *A. gracilipes* and the mean body size of the opponent species was determined with secondary order polynomial regression. Exponential regression model was used to determine the relationship between the mean frequency of the behavioral index 5 of *A. gracilipes* and the mean percentage of mortality of the opponent species. All analyses were performed using CurveExpert version 1.3 (Daniel Hyams, Hixson, TN). The

Table 1. Measurement (in millimeters) of body size (mandible tip to gaster tip), head width, and mandible width of the worker ants of *A. gracilipes* and the sympatric ant species used in this study

Species	Mean ± SEM body size (n = 10)	Mean ± SEM head width (n = 10)	Mean ± SEM mandible width (n = 10)
M. orientale	1.41 ± 0.02	0.29 ± 0.00	N.A.[c]
M. floricola	1.67 ± 0.01	0.31 ± 0.00	N.A.
M. destructor	2.07 ± 0.04[a]/2.81 ± 0.05[b]	0.45 ± 0.01[a]/0.60 ± 0.01[b]	0.30 ± 0.02[a]/0.72 ± 0.02[b]
M. pharaonis	2.25 ± 0.04	0.43 ± 0.01	0.43 ± 0.01
P. parva	1.99 ± 0.04[a]/2.87 ± 0.06[b]	0.46 ± 0.00[a]/0.88 ± 0.01[b]	0.49 ± 0.03[a]/0.76 ± 0.01[b]
Crematogaster sp.	2.38 ± 0.04	0.47 ± 0.01	0.28 ± 0.01
S. geminata	3.58 ± 0.07[a]/4.93 ± 0.07[b]	0.68 ± 0.01[a]/1.32 ± 0.11[b]	0.90 ± 0.07[a]/1.28 ± 0.07[b]
T. indicum	1.81 ± 0.04	0.43 ± 0.00	N.A.
T. melanocephalum	1.80 ± 0.03	0.40 ± 0.00	N.A.
T. butteli	2.93 ± 0.05	0.56 ± 0.01	0.82 ± 0.01
D. thoracicus	3.57 ± 0.05	0.93 ± 0.02	1.17 ± 0.03
P. longicornis	2.76 ± 0.05	0.52 ± 0.00	0.89 ± 0.02
O. smaragdina	8.75 ± 0.19	1.72 ± 0.04	2.76 ± 0.06
Camponotus sp.	7.30 ± 0.11	1.26 ± 0.02	1.91 ± 0.03
A. gracilipes	4.31 ± 0.08	0.66 ± 0.01	1.06 ± 0.05
T. rufonigra	10.15 ± 0.11	1.79 ± 0.02	1.47 ± 0.08

[a] Minor worker,
[b] Major worker.
[c] N.A., not available.

frequencies of each behavioral index among different ant species were analyzed with Kruskal–Wallis (KW) one-way analysis of variance (ANOVA), and means were separated using KW multiple range test. The analyses were done using Statistix version 7 (Analytical Software, Tallahassee, FL).

Intraspecific Aggression. Tests were carried out between the three populations of *A. gracilipes* described above by using individual and group assays. Individual assays were conducted between MC and MTG, BM and MTG, and BM and MC. Ten replicates were conducted for each pair. To enable identification of the populations, before the test, the insects from one population were given water containing a red food dye (Bush Boake Allen Ltd, London, United Kingdom). The red dye had been tested earlier and was found not to influence the nestmate recognition behavior among the worker ants. No significant difference ($P < 0.001$) in behavior was found between the dyed and undyed individuals toward nestmates and non-nestmates (K.F.C., unpublished data). For the group assay, the same procedure described above in interspecific aggression test was used on similar pairings of groups of the three populations: between MC and MTG, between BM and MTG, and between BM and MC.

The frequencies of each behavioral index between two opposing populations of *A. gracilipes* in individual assays were analyzed with Wilcoxon signed rank test using Statistix version 7 (Analytical Software, Tallahassee, FL).

Results

Table 1 showed the measurement of body size, head width, and mandible width of the 16 species of ants used in this study. We were unable to measure the mandible width of four ant species (*M. orientale, M. floricola, T. indicum,* and *T. melanocephalum*) due to the extremely small mandibles of these species.

Interspecific Aggression. *Individual Assay.* The individual assay demonstrated *A. gracilipes*'s aggressive behavior toward most of the opponent species. They were aggressive toward *S. geminata, P. longicornis, P. parva* (major workers), *O. smaragdina, Camponotus* sp., and *T. rufonigra,* with mean frequency of the most aggressive behavior (behavioral index 5) of 4.7–9.4 (Table 2). They are less aggressive to *Crematogaster* sp.; *M. destructor;* the major workers of *P. parva, T. butteli, D. thoracicus, T. indicum,* and *T. melanocephalum; P. parva* (minor workers); *M. floricola;* and *M. pharaonis. A. gracilipes* showed no aggression toward *M. orientale.*

The tactic most commonly used by *A. gracilipes* when interacting with its opponent species was to lift its gaster forward to spray formic acid (O'Dowd et al. 2003). To do so, it would first grasp a body part of its opponent. This tactic was very effective against the bigger opponent species such as *O. smaragdina, Campontus* sp., and *T. rufonigra.* The ant would usually spray toward the facial area on the head of the opponent species. O'Dowd et al. (2003) reported that *A. gracilipes* overwhelmed the red land crabs in Christmas Island by spraying formic acid toward the eyes and mouthparts of the crabs, and the crabs will succumb to the attack within 48 h. From our observations, upon being sprayed, the opponent became irritated and tried to clean the affected portion of the body by grooming or rubbing it with its legs. *A. gracilipes* also would clean its gaster after spraying the formic acid, because the formic acid would kill the insect as well if it came into contact with it for many times. Brian (1983) reported that formic acid is effective irritant against both arthropods and mammals.

Confrontations between *A. gracilipes* and *T. rufonigra,* and between *A. gracilipes* and *O. smaragdina* resulted in greater mortality when compared with *A. gracilipes*'s interaction with the other species (Table 2). Compared with other species, both of these op-

Table 2. Mean frequency for each behavioral indices of *A. gracilipes* when interacting with the opponent ant species under 15-min individual assay

Species	Mean frequency for each behavioral index ± SEM[a]						Ratio of *A. gracilipes* win:lose:both survive:both dead
	0	1	2	3	4	5	
M. orientale	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0:0:10:0
M. pharaonis	0.00 ± 0.00a	0.10 ± 0.10a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.10 ± 0.67abcd	0:0:10:0
M. floricola	0.00 ± 0.00a	0.30 ± 0.20a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.90 ± 0.59abcd	0:0:10:0
Crematogaster sp.	0.00 ± 0.00a	0.50 ± 0.27a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10ab	0:0:10:0
M. destructor							
Minor worker	0.00 ± 0.00a	0.40 ± 0.20a	0.30 ± 0.30a	0.40 ± 0.27a	2.80 ± 0.95a	1.10 ± 0.38abcd	1:0:9:0
Major worker	0.00 ± 0.00a	0.90 ± 0.50a	0.00 ± 0.00a	0.70 ± 0.33a	2.90 ± 1.12a	2.00 ± 0.97abcd	3:0:7:0
P. parva							
Minor worker	0.00 ± 0.00a	1.20 ± 0.71a	0.00 ± 0.00a	0.10 ± 0.10a	0.00 ± 0.00a	1.10 ± 0.53abcd	2:0:8:0
Major worker	0.00 ± 0.00a	1.40 ± 0.52a	0.00 ± 0.00a	0.10 ± 0.10a	0.20 ± 0.20a	5.20 ± 1.62abcd	2:0:8:0
S. geminata							
Minor worker	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.10 ± 0.8a	0.80 ± 0.39a	9.40 ± 2.57d	7:0:3:0
Major worker	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.20 ± 0.80a	1.30 ± 0.45a	4.70 ± 0.92bcd	3:1:6:0
T. indicum	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10a	0.80 ± 0.39a	0.40 ± 0.22abc	1:0:9:0
T. melanocephalum	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.40 ± 0.31a	2.70 ± 1.26a	0.60 ± 0.27abcd	1:0:9:0
T. butteli	0.00 ± 0.00a	0.10 ± 0.10a	0.00 ± 0.00a	0.10 ± 0.10a	2.20 ± 1.45a	7.20 ± 1.65cd	8:0:2:0
D. thoracicus	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10a	1.60 ± 0.64a	0.20 ± 0.13a	1.10 ± 0.23abcd	6:0:4:0
P. longicornis	0.00 ± 0.00a	0.20 ± 0.20a	0.10 ± 0.10a	0.30 ± 0.30a	2.40 ± 0.96a	7.60 ± 2.36cd	8:0:2:0
O. smaragdina	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.70 ± 0.67a	0.40 ± 0.31a	5.90 ± 1.11cd	4:2:1:3
Camponotus sp.	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.50 ± 0.22a	3.60 ± 0.88a	5.70 ± 1.71abcd	1:0:9:0
T. rufonigra	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10a	0.30 ± 0.30a	1.20 ± 0.99a	6.50 ± 2.22bcd	4:4:0:2

[a] Means followed by different letters within the same column are significantly different ($P < 0.05$; Kruskal–Wallis multiple range test).

ponent species were more tolerant to formic acid. The seizing and biting strategies used by *A. gracilipes* failed because these opponent species have relatively hard bodies. However, *T. rufonigra* and *O. smaragdina* attacked *A. gracilipes* by biting. They have very strong mandibles and were able to macerate *A. gracilipes.* The outcome of the interaction often resulted high mortality for both species.

Interspecific aggression, however, did not always result in mortality. Although *A. gracilipes* was highly aggressive to *Camponotus* sp., this species was not killed. Aggressive behavior lasted only 2–3 min after the ants were introduced into the test arena. *Camponotus* sp. did not demonstrate any aggressive behavior toward *A. gracilipes.* In the test, only one case of *Camponotus* sp. being killed by *A. gracilipes* was recorded (Table 2).

Some species, such as *T. butteli, D. thoracicus,* and *P. longicornis,* were very susceptible to the formic acid spray. In general, *A. gracilipes* won the interaction with these species (Table 2). It also could easily kill *S. geminata* with formic acid spray. *S. geminata* would raise up its abdomen for defense and sting whenever possible, but this tactic usually failed because *A. gracilipes* would avoid the sting by quickly moving away. In the interaction between *D. thoracicus* and *A. gracilipes, D. thoracicus* usually became immobile and opened its mandibles widely for defense. *T. butteli* also would become immobile. In contrast, *P. longicornis* ran to avoid contact with *A. gracilipes,* but upon meeting one another, it will seize the legs and antennae of *A. gracilipes.*

The interspecific interactions between *A. gracilipes* and *Crematogaster* sp., *M. destructor, T. butteli, T. indicum,* and *T. melanocephalum* were less aggressive. *A. gracilipes* avoided these species, especially *T. indicum* and *T. melanocephalum.* In some cases, *A. gracilipes* responded to these species by spraying formic acid, but this tactic normally failed because these small species were difficult to grasp. *A. gracilipes* showed limited aggression toward the minor worker of *P. parva, M. pharaonic,* and *M. floricola* and no aggression to *M. orientale.* It normally jerked back or walked away when it met these species, and minimal mortality was encountered (Table 2). *M. pharaonis, M. floricola,* and *M. orientale* raised their abdomen for defense, whereas the minor workers of *P. parva* remained immobile after being introduced into the test arena.

Group Assay. Table 3 shows the mortality of both *A. gracilipes* and opponent species during the 24-h interaction. Approximately 40% of the tested species lost in the group interaction with *A. gracilipes;* specifically, *M. destructor* and the major workers of *P. parva, S. geminata, D. thoracicus, P. longicornis,* and *O. smaragdina.* As observed in the individual assay, in this study *P. longicornis* and *D. thoracicus* were affected by formic acid and they were killed by *A. gracilipes.* For the species with dimorphic workers, such as *M. destructor, P. parva,* and *S. geminata, A. gracilipes* was more aggressive toward the major workers compared with the minor workers, similar to the observation made in individual interaction test. When *A. gracilipes* and *O. smaragdina* were placed together, the level aggression was high, resulting in high mortality of both species at 85 and 76%, respectively (Table 3). *O. smaragdina* normally raised its abdomen and secreted mandibular gland pheromones to alert or attract the other nestmates (Brian 1983), so that they could locate *A. gracilipes* and induce biting. *A. gracilipes* also was killed at a higher rate when interacting with *T. rufonigra,* with >70% mortality observed in these interactions.

Table 3. Mean percentage of mortality of both *A. gracilipes* and its opponent species after 24-h group assay

Species	Mean % mortality of opponent species ± SEM[a]	Mean % of mortality of *A. gracilipes* ± SEM[a]
M. orientale	14.00 ± 2.67 (LS)	1.00 ± 1.00 (LS)
M. pharaonis	70.00 ± 7.30 (S0	8.00 ± 4.67 (LS)
M. floricola	3.00 ± 2.13 (LS)	0.00 ± 0.00 (NS)
Crematogaster sp.	50.00 ± 5.58 (S)	0.00 ± 0.00 (NS)
M. destructor	92.00 ± 5.12[b] (VS) / 100.00 ± 0.00[c] (VS)	3.00 ± 2.13[b] (LS) / 3.00 ± 2.13[c] (LS)
P. parva	10.00 ± 4.22[b] (LS) / 84.00 ± 6.70[c] (VS)	1.00 ± 1.00[b] (LS) / 2.00 ± 1.33[c] (LS)
S. geminata	85.00 ± 6.87[b] (VS) / 100.00 ± 0.00[c] (VS)	34.00 ± 13.10[b] (MS) / 2.00 ± 1.33[c] (MS)
T. indicum	18.00 ± 6.11 (LS)	3.00 ± 2.13 (LS)
T. melanocephalum	18.00 ± 8.00 (LS)	5.00 ± 2.24 (LS)
T. butteli	68.00 ± 9.52 (S)	1.00 ± 1.00 (LS)
D. thoracicus	100.00 ± 0.00 (VS)	3.00 ± 3.00 (LS)
P. longicornis	100.00 ± 0.00 (VS)	0.00 ± 0.00 (NS)
O. smaragdina	85.00 ± 4.77 (VS)	76.00 ± 7.48 (VS)
Campontus sp.	53.00 ± 9.55 (S)	7.00 ± 2.13 (LS)
T. rufonigra	58.00 ± 8.00 (S)	72.00 ± 11.53 (S)

[a] 0%, not susceptible (NS); >0–<25%, less susceptible (LS); 25–<50%, moderately susceptible (MS); 50–<75%, susceptible (S); and 75–100%, very susceptible (VS).
[b] Minor worker.
[c] Major worker.

Interaction between *A. gracilipes* and *M. pharaonis,* and *Crematogaster* sp. resulted in 70 and 50% mortality, respectively, of these opponent species. This result was unexpected because *A. gracilipes* was less aggressive toward these two species in the individual assays. However, Retana and Cerdá (1995) showed that *Camponotus foreli* Emery was a nonaggressive species in an individual test but was the second most aggressive species in group tests. This result could be due to the space constraint in the group test, in which *A. gracilipes* had a greater chance of meeting the opponent species than in the individual test. In contrast, the mortalities of *T. indicum* and *T. melanocephalum* were lower than that of *M. pharaonis* and *Crematogaster* sp. in group tests with *A. gracilipes* (Table 3). From our observation, *A. gracilipes* moved away from *T. indicum* and *T. melanocephalum* when they met, and *T. indicum* and *T. melanocephalum* congregated in one location within the test arena.

Relationship Between Aggression Frequency of *A. gracilipes* and the Size of Opponent Species. In individual assays, we found that the level of aggression of *A. gracilipes* increased with the body size (Fig. 1), head width ($y = 5.827x - 9.474$. $r^2 = 0.8341$, $P < 0.0001$, where y is mean frequency of behavioral index 5 of *A. gracilipes,* and x is mean head width of opponent species), and mandible width ($y = 3.490x + 6.676$. $r^2 = 0.6079$, $P < 0.001$, where y is mean frequency of behavioral index 5 of *A. gracilipes*, and x is mean mandible width of opponent species) of the opponent species. However, group assays showed that mortality of *A. gracilipes* has a significant ($P < 0.05$) positive relationship with body size of opponent species (Fig. 2), with r^2 values >0.7. The relationship between

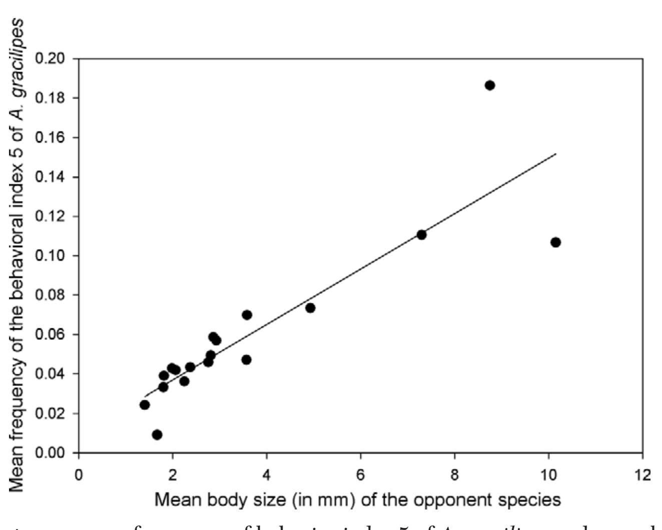

Fig. 1. Relationship between mean frequency of behavior index 5 of *A. gracilipes* and mean body size (millimeters) of opponent species. Unbroken line represents the best fit line ($y = 1.065x - 0.480$. $r^2 = 0.7692$, $P < 0.0001$).

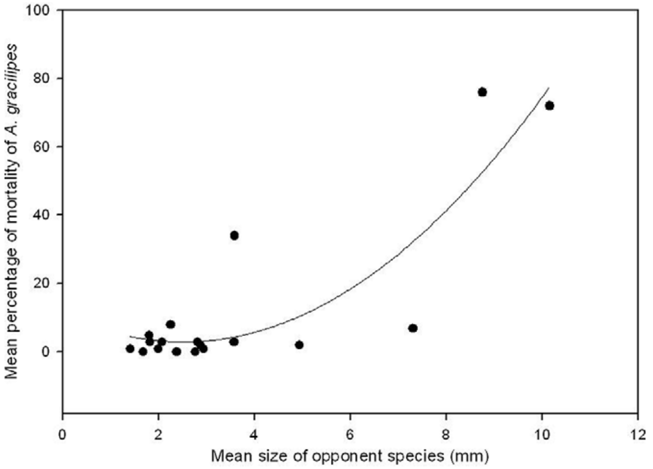

Fig. 2. Relationship between mean percentage of mortality of *A. gracilipes* and the mean body sizes of opponent species. Unbroken line represents the curve of best fit (second order polynomial function: $y = 1.278x^2 - 6.439x + 11.013$. $r^2 = 0.766$, $P < 0.0001$).

mortality of *A. gracilipes* (y) and mean head width of the opponent species (x) is a second order polynomial function: $y = 59.419x^2 - 81.766x + 27.080$, $r^2 = 0.776$, $P < 0.0001$. The mandible width of the opponent species (x) also significantly ($P < 0.05$) affected the mortality rate of *A. gracilipes* (y) ($y = 8.505x^2 - 2.338x + 0.401$, $r^2 = 0.501$, $P < 0.05$). This finding suggested that size is a determining factor in the success of interspecific aggression in ants. Retana and Cerdá (1995) also reported that interspecific aggressiveness of ants was directly related to size of species in the group test.

Exponential regression analysis revealed that a significant ($P < 0.05$) positive relationship between frequency of aggression of *A. gracilipes* in individual interaction and the mortality of the opponent species in group tests (Fig. 3), which indicated that the higher the frequency of aggression in *A. gracilipes*, the greater the chance that the opponent species will be killed by *A. gracilipes.*

Intraspecific Interaction. Individual assays showed that different populations of *A. gracilipes* were highly aggressive toward one another (Table 4). MTG was highly aggressive toward both BM and MC ants. These populations were not unicolonial and demonstrated intraspecific aggression toward one another. The results also indicated that there are varying degrees in the frequency of each aggression behavior between different populations tested.

In group assays, both MC and BM populations were very susceptible to mortality by the MTG (>80% mortality; Table 5). In contrast, the interaction between the MC and BM populations was less aggressive. BM killed only 20% of the MC population and MC killed

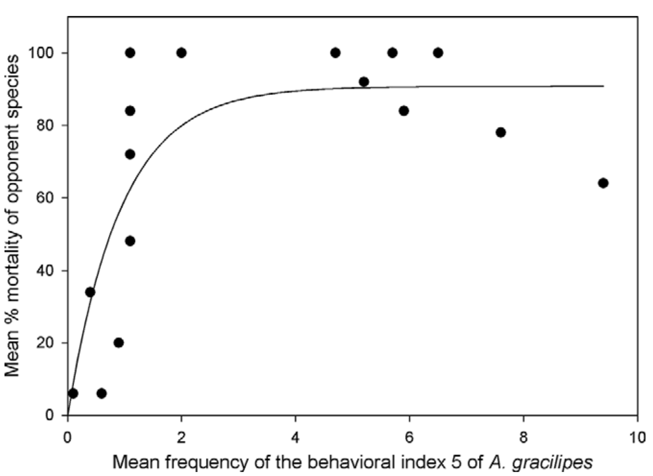

Fig. 3. Relationship between mean percentage of mortality of the opponent species and mean frequency of behavioral index 5 of *A. gracilipes*. Unbroken line represents the curve of the best fit (exponential model: $y = 90.777[1 - e^{-1.0612x}]$, $r^2 = 0.6411$, $P < 0.05$).

Table 4. Mean frequency for each behavioral index of *A. gracilipes* from different populations in 15-min individual assay

Pop Interaction[a]	Mean frequency for each behavioral index ± SEM[b]					
	0	1	2	3	4	5
MC vs.						
MTG	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.90 ± 0.23a	0.20 ± 0.13a	1.10 ± 0.23a
BM	0.00 ± 0.00a	0.00 ± 0.00a	0.30 ± 0.15a	0.30 ± 0.15b	0.20 ± 0.13a	0.30 ± 0.15b
MTG vs.						
MC	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10a	0.50 ± 0.22a	0.70 ± 0.26a	2.70 ± 0.50a
BM	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.30 ± 0.15a	0.60 ± 0.16a	0.90 ± 0.28b
BM vs.						
MTG	0.00 ± 0.00a	0.10 ± 0.10a	0.00 ± 0.00a	0.80 ± 0.20a	0.50 ± 0.17a	0.50 ± 0.17a
MC	0.00 ± 0.00a	0.00 ± 0.00a	0.10 ± 0.10a	0.80 ± 0.13a	0.30 ± 0.15a	0.60 ± 0.16a

[a] MC, Minden Campus; MTG, Matang; and BM, Bukit Mertajam.
[b] Means followed by different letters within the same column are significantly different ($P < 0.05$; Wilcoxon signed rank test).

none of the workers of BM. Aggression between these two populations occurred only at the beginning of the test and lasted for 20 min. After that, they congregated in one location in the test arena, which could indicate territoriality. It is possible that the size of the territory is related to the level of aggression of the population (i.e., the more aggressive the population, the larger the territory). The results from the group tests corresponded with those from the individual interaction tests; MTG were highly aggressive to both MC and BM. This result also confirms that intraspecific aggressive behavior occurs in *A. gracilipes.*

Discussion

Interspecific competition is a nearly ubiquitous feature of ant communities (Hölldobler and Wilson 1990). Ants compete fiercely for the same resources (e.g., food and space). This competition often leads to agonistic behavior, such as fights, escape, and submission (Buschini and Leonardo 2001). Different species of ants exhibit different levels of aggression (Horvitz and Schemske 1984). The common weapons used in defense, attack, or both are stings, toxic smears, and repellents (Howse 1983, Hölldobler and Wilson 1990). Howse (1983) reported that members of the subfamily Formicinae do not have a sting; they usually bite and spray toxic secretions. This behavior was evidently observed in *A. gracilipes* in this study that bites and sprays formic acid. However, *O. smaragdina, P. longicornis,* and *Camponotus* sp. seldom use chemical defense strategies. Instead, *O. smaragdina* macerates its opponents into pieces by using its strong mandibles.

For small ants, due to their inferior size, they use other defense techniques: They can be highly mobile, develop chemical repellents that can act at a distance, abandon resistance in the face of attack, or become immobile (Howse 1983). In this study, all these behaviors were observed in the *T. indicum* and *T. melanocephalum* interactions with *A. gracilipes.* These two species also were able to secrete toxic materials to repel *A. gracilipes.*

In addition, other small ants in this study (e.g., *Monomorium* spp. and *P. parva*) remained immobile when they came into contact with the bigger ants (i.e., *A. gracilipes*). Retana and Cerdá (1995) suggested that tempo (speed of movement) also was related to the defense strategy of ants. Low-tempo species (*M. orientale, M. pharaonis, M. floricola, Crematogaster* sp., *M. destructor, P. parva, S. geminata, T. butteli,* and *D. thoracicus*) usually use an immobile strategy to avoid attacks, whereas high-tempo species (*T. indicum, T. melanocephalum, P. longicornis, O. smaragdina, Camponotus* sp. and *T. rufonigra*) use an escape strategy when coming into contact with a more aggressive species. In this study, *P. longicornis* and *Camponotus* sp. ran very fast to avoid coming into contact with *A. gracilipes.* However, an escape strategy would not be effective if the space of test arena was limited, and this would induce an attack on their opponents. Some *Camponotus* spp. workers responded to contact with *A. gracilipes* with submissive behavior, in which they folded into a pupa-like position and became motionless, and *A. gracilipes* carried them away with their mandibles. Smaller ants such as *M. orientale* and *M. floricola* grouped together and remained motionless.

The current study found that the level of aggression of *A. gracilipes* increased with the size of the opponent species. Similar observations have been reported by other researchers. Dreisig (1988) reported that aggression or competitive ability of ants was correlated with their body size. Jutsum (1979) found that worker size difference was an important criterion in determining the interspecific aggressive interaction of leaf-cutting ants.

We found intraspecific aggression between *A. gracilipes* from different localities in Malaysia. This did not concur well with that reported by Abbott (2005) that *A. gracilipes* from isolated nests on Christmas Island did not display aggressive behavior. The contrasting results in both studies could be due to the

Table 5. Mortality of *A. gracilipes* from different populations after 24-h group assay

Interaction	Mean % mortality ± SEM[a]		
	MC	BM	MTG
MC vs. MTG	86.00 ± 7.48 (VS)		4.00 ± 2.45 (LS)
BM vs. MTG		80.00 ± 15.49 (VS)	30.00 ± 5.48 (MS)
BM vs. MC	22.00 ± 9.70 (LS)	0.00 ± 0.00 (NS)	

[a] 0%, not susceptible (NS)' >0–<25%, less susceptible (LS); 25–<50%, moderately susceptible (MS); 50–<75%, susceptible (S); and 75–100%, very susceptible (VS).

greater genetic diversity of the *A. gracilipes* populations found in Malaysia compared with those in Christmas Island. Abbott (2005) speculated that a single related supercolony may occupy the entire Christmas Island. Ants in introduced regions often display low levels of intraspecific aggression, due to decreased genetic variation at loci associated with kin recognition (Suarez et al. 1999); in contrast, ants in their native range have greater genetic diversity, which might account for the higher levels of aggression observed in this study. Although the native range of *A. gracilipes* is unknown, it was proposed that it could be Africa, or the moist tropical regions of the Orient (e.g., southern India, Sri Lanka, some islands in the Indian Ocean, and Southeast Asia) (Wetterer 2005). The discovery of a myrmecophilous cricket *Myrmecophilus leei* Kistner & Chong inside *A. gracilipes* nests in Malaysia (Kistner et al. 2007) may further reinforce the speculation that *A. gracilipes* is native to Southeast Asia.

Over the past several years, we have observed increasing infestation of *A. gracilipes* in Malaysia. In the Universiti Sains Malaysia Minden campus, *A. gracilipes* is presently the most important pest ant species after *P. longicornis* and *T. melanocephalum* (C.Y.L., unpublished data). This was not the situation in an earlier survey where *M. destructor*, *P. longicornis,* and *P. parva* were the three most dominant species (Lee 2002). The reason contributing to the rising importance of *A. gracilipes* is unknown and warrants further investigation. However, this species has reduced the ecological dominance of these three sympatric species as pest ants.

Laboratory assays have been widely used to study the inter- and intra-aggression of pest ants, either by using individual (Retana and Cerdá 1995; Tsutsui et al. 2000; Abbott 2005; Kirschenbaum and Grace 2008ab), group (Retana and Cerdá 1995; Chen and Nonacs 2000; Kirschenbaum and Grace 2008a,b), or colony assays (Silverman and Liang 2001). Although observations made in the laboratory may not necessarily reflect the actual aggressiveness of the ants in the field (Le Moli and Parmigiani 1981, Le Moli et al. 1984, Roulston et al. 2003), Retana and Cerdá (1995) clarified that they are useful in providing insights into the methods used and the success obtained during the interactions of these species. Controlled laboratory experiments also permit analysis of the potential aggressiveness of ant species in interspecies interaction without taking into account the social features that may mask specific aggression behavior.

In summary, *A. gracilipes* exhibited aggressive behavior against most of the sympatric ant species tested, especially toward those with larger body size. Only *O. smaragdina* and *T. rufonigra* were able to compete effectively against this species during group interactions. In contrast, smaller species such as *M. orientale* and *M. floricola* survived both individual and group assays by remaining immobile. Aggressive behavior and mortality of *A. gracilipes* were significantly influenced by the body size, head width, and mandible width of opponent species. In the intraspecific aggression tests, different populations of *A. gracilipes* from different localities in Malaysia demonstrated aggressive behavior toward one another.

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