

COMPARATIVE STUDY ON THE ANTHROPOGENIC INFLUENCE ON DISTRIBUTION
OF THE LONG-LEGGED ANT AND ARGENTINE ANT

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A capstone project submitted for Graduation with University Honors

May 6, 2022

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University of California, Riverside

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ABSTRACT

The long-legged ant (*Anoplolepis gracilipes*) and the Argentine ant (*Linepithema humile*) are highly invasive tramp ants well-known for their deleterious effects on native ecosystems where they have been introduced. While tramp ants, by definition, are associated with human activity, research on how different intensities of human activity affect ant distribution is limited. This study investigated how anthropogenic activities affected the distribution of *A. gracilipes* in Penang Island (Malaysia) and *L. humile* in Riverside, California (USA). Three study sites were selected for each species. Each site contained four sub-locations corresponding to four different levels of human activity (low, moderate, high, and very high) as determined by the average number of passersby observed over 30 minutes. Baited index cards were placed at each sub-location to evaluate ant abundance and distribution. Subsequently, general linear modeling with Poisson regressions was used to assess the relationship between human activity and ant abundance. The results demonstrate that *A. gracilipes* distribution patterns were consistent with the previous study, with ant abundance being highest in areas of moderate human activity. In contrast, *L. humile* abundance was not significantly correlated with human activity.

ACKNOWLEDGMENTS

Firstly, I would like to thank my faculty mentor, Dr. Chow-Yang Lee, whose constant support and guidance have allowed me to successfully complete this project, which was partially undertaken remotely while we were on opposite sides of the globe in the middle of a raging pandemic. His mentorship has been invaluable in molding my personal growth as an aspiring researcher. Besides that, I am grateful to Dr. Shu-Ping Tseng of the Lee Lab at UC Riverside and Dr. Ming-Chung Chiu of the National Changhua University of Education, Taiwan, for their assistance with the statistical analysis and modeling. I would also like to give special thanks to my parents, Chee Foo Lum and Ai Chin Tan, for never failing to support me in all my endeavors and especially to my mother for often accompanying and driving me to the various surveyed locations. The UCR Undergraduate Education Mini-Grant Program and the Carl Strom/Western Exterminator Company Scholarship provided funding for this project.

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INTRODUCTION

Ants (Hymenoptera: Formicidae) are some of the most frequently encountered groups of insects within urban environments. Many common ant species found in human-altered environments are “tramp ants,” which have invaded numerous regions worldwide outside of their native range due to accidental introductions along international trade routes. Indeed, the primary unifying characteristic among tramp ant species is that they are strongly anthropophilic, reliant on humans for long-distance dispersal, and prefer to nest close to human habitation. Still, in general, they display the other following traits: small and sterile workers, polygyny, unicoloniality, and colony reproduction via budding from the reduced or complete absence of nuptial flight (Passera, 1994). Among tramp ants, several species are of significant global concern due to their deleterious effects on native ecosystems, including the two species investigated in this study, the yellow crazy ant (*Anoplolepis gracilipes* [Fr. Smith]) and the Argentine ant (*Linepithema humile* Mayr), which are widely considered to be significant pests (Wetterer, 2015). *A. gracilipes* now occupies various tropical and subtropical areas in Asia and Oceania and has been introduced to many islands of the Indian and Pacific oceans (Lee & Yang, 2022). On the other hand, *L. humile* has invaded many subtropical and temperate regions worldwide, especially Mediterranean climate areas (Silverman and Brightwell, 2008). Its current introduced range encompasses the United States, Japan, France, Italy, Australia, New Zealand, etc.

Both *A. gracilipes* and *L. humile* are associated with many changes in invaded habitats. Many of these changes can be attributed to the high interspecific aggression often exhibited by tramp ant species (Passera, 1994). In large numbers, they can outcompete native ants and monopolize food sources through both exploitation and interference competition (Human and Gordon, 1996; Drescher et al., 2011). Areas with high populations of *A. gracilipes* have lower

native ant diversity (Bos et al., 2008), while invasions of *L. humile* in California were reported to have long-term effects on native ant richness and species composition that have endured for at least 30 years (Menke et al., 2018). Furthermore, both species have been associated with population declines of other arthropods and may even negatively affect higher organisms like vertebrates through nesting failures, displacement of prey, or direct extirpation (Holway et al., 2002; Lee & Yang, 2022). In agricultural systems, they protect mutualistic sap-feeding hemipterans feeding on crops and facilitate outbreaks of these pests (Holway et al., 2002; Lee & Yang, 2022). Probably the most infamous case of severe *A. gracilipes* invasion was documented on Christmas Island, where the ants were responsible for an ecological “meltdown” by killing large numbers of endemic red crabs and causing the death of mature trees, leading to large-scale changes in forest structure (O’Dowd et al., 2003).

As tramp ants, the anthropophilic tendencies of both *A. gracilipes* and *L. humile* are well-documented. However, studies on how different intensities of human activity impact the distribution of tramp ants are very limited. For *A. gracilipes*, a recent study conducted in Taiwan found that the highest abundance of ants was detected in areas of intermediate human disturbance (Lee et al., 2021). Similar distribution patterns have been observed for *L. humile* in the San Francisco Bay Area. An extremely high abundance of ants was recorded in semi-natural areas, much higher than urban, natural, and agricultural sites (Vonshak and Gordon, 2015). However, it is uncertain if identical distribution patterns can also be observed across different geographical areas for both species. This project will attempt to fill some of these gaps in our knowledge and investigate how *A. gracilipes* and *L. humile* abundance are correlated with human activity in Penang Island (Malaysia) and Riverside, California (USA), respectively. From what we know, many studies have determined that *A. gracilipes* are highly abundant in plantations and

agroforests in Southeast Asia while being absent from natural forests (Bos et al., 2008; Brühl and Eltz, 2010; Konopik et al., 2014) or much less abundant in rainforest remnants (Asfiya et al., 2015). Within these plantations and agroforests, *A. gracilipes* are often notable for being the most abundant ant species (Bos et al., 2008; Brühl and Eltz, 2010; Asfiya et al., 2015). Likewise, in southern California, Menke et al. (2007) found a strong positive correlation between *L. humile* occurrence and closeness to urban areas, while Staubus et al. (2019) found *L. humile* to be very common within suburban areas, moderately common in native sage scrub and least common in non-native grassland. On a global scale, current models suggest that occurrences of *L. humile* are most dependent on suitable climatic conditions and human-modified habitats (Roura-Pascual et al., 2011).

Based on the available evidence, we hypothesize that the highest abundance of *A. gracilipes* and *L. humile* will be observed in moderate or high human activity areas. Several field surveys were conducted to ascertain the relationship between the intensity of human activity and ant abundance to evaluate this hypothesis. The findings from these studies would help us identify potential mechanisms responsible for high tramp ant abundance and direct targeted efforts to locate population reservoirs of ants that serve as a source of future invasions.

MATERIALS AND METHODS

The study area for *A. gracilipes* was designated as Penang Island (05° 25' N, 100° 16' E), located off the northwestern coast of Peninsular Malaysia in the Strait of Malacca, encompassing an area of 293 km² or 113 sq mi. Due to its equatorial location, Penang Island possesses a tropical climate with abundant sunshine, uniformly high temperatures, humidity, and rainfall year-round. However, a distinct dry season is still observed from early November to late March.

While much of the island has undergone urban, suburban, and agricultural development, a substantial portion remains naturally forested. For *L. humile*, the main campus of the University of California, Riverside (33° 58' N, 117° 19' W) was utilized as the study area. The UCR main campus is located in the city of Riverside in western Riverside County, covering an area of 4.50 km² or 1,112 acres. The city of Riverside experiences a Mediterranean climate with hot, dry summers and mild winters. Almost all precipitation falls between November and April, with February being the wettest month.

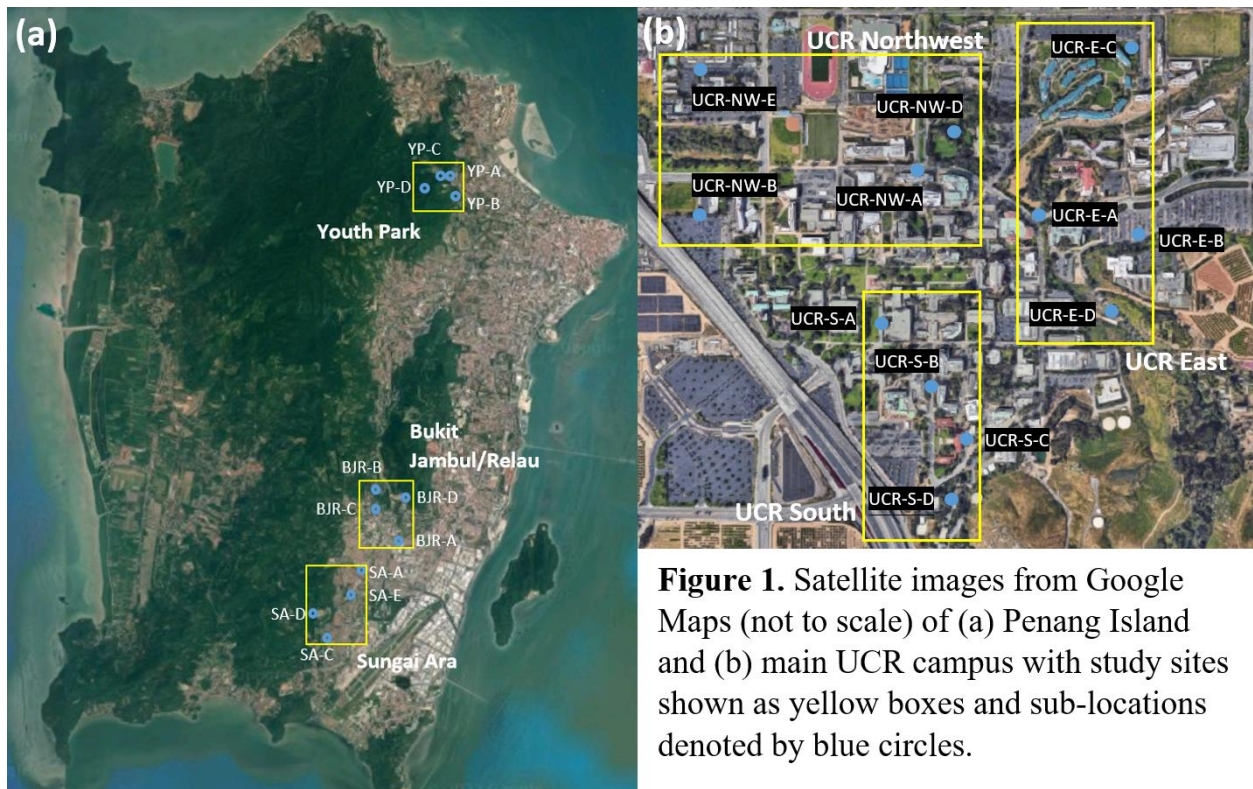


Figure 1. Satellite images from Google Maps (not to scale) of (a) Penang Island and (b) main UCR campus with study sites shown as yellow boxes and sub-locations denoted by blue circles.

Three study sites with a gradient of human activity (i.e., going from high-activity to low-activity areas) were selected for both study areas. On Penang Island, the three study sites chosen were Youth Park, Sungai Ara, and Bukit Jambul/Relau (Fig. 1a). Meanwhile, the three study sites on the main campus of UCR were created by dividing the area into three zones: UCR Northwest, UCR South, and UCR East (Fig. 1b). Four suitable sub-locations within each study

site where *A. gracilipes* or *L. humile* were sighted or likely to appear were selected. Each corresponds to levels one to four of human activity: low, moderate, high, and very high. Human activity within the sub-locations was recorded by counting the number of passersby observed over thirty minutes during the sampling period, with the average number of passersby being used to classify each sub-location into one of the four categories as mentioned above: low = 0.0–7.9 passersby; moderate = 8.0–24.9 passersby; high = 25.0–59.9 passersby; very high = ≥ 60.0 passersby. Due to the Full Movement Control Order (FMCO) or shelter-in-place due to the COVID-19 pandemic resulting in reduced numbers of passersby, a sub-location with very high human activity within the Youth Park site could not be found, so instead, an additional sub-location with high human activity was surveyed. Ten sampling points were set up in each sub-location to investigate the abundance and distribution of the tramp ant species of interest. The sampling points were spread apart by a distance of at least 5 m from each other and placed adjacent to ant trails or where worker ants were expected to forage. Baited 3” x 5” index cards were deployed at these sampling points, each with half a teaspoon of honey and peanut butter serving as carbohydrate and protein/lipid sources, respectively. Thirty minutes after deployment, photos of the index cards were taken with a digital camera (Samsung WB850F), and temperature and relative humidity readings were taken with a simple analog thermometer and hygrometer. These surveys were conducted between 8:00 a.m. and 10:00 a.m., when the activity of *A. gracilipes* and *L. humile* is known to be high (Markin, 1970; Chong and Lee, 2009). In addition, the surveys were repeated for a total of six or seven days per sub-location, on days with no inclement weather such as rain. Ant abundance and distribution was counted using the digital images to obtain an abundance score according to the following categories: 0 = no ants; 1 = 1–5 ants; 2 = 6–20 ants; 3 = 21–50 ants; 4 = 51–100 ants; 5 = > 100 ants. According to the sampling

date, the abundance scores were then pooled and averaged for each sub-location. Approximately five individual specimens of every ant species encountered at each sub-location were collected and preserved in 70-75% ethanol. Subsequently, these specimens were identified to species level wherever possible, or at least to genus level. Identification keys by Na and Lee (2001) and Fayle et al. (2014) were used to identify specimens collected from Penang, while Fisher and Cover (2007) were used to identify specimens collected from Riverside. Keys provided by AntWiki (2022) were also used to refine species identification further when necessary.



Figure 2. Baited index cards provisioned with honey and peanut butter, with (a) showing the feeding behavior of *A. gracilipes* workers in Penang Island and (b) showing the feeding behavior of *L. humile* workers in Riverside.

Generalized linear models (GLMs) assuming a Poisson distribution for average ant abundance score were fitted using a log link function to analyze the factors that could potentially be important in influencing tramp ant distribution. The independent variables assessed were the natural logarithm of (no. of passersby + 1) and its quadratic term ($\log(\text{no. of passersby} + 1)^2$), temperature, relative humidity. Initially, all of the independent variables were included to construct the whole model, then different combinations of independent variables were used to determine the best model. The final model or optimum approximating model was the model with the lowest Akaike's information criterion (AIC) value. Models with an AIC difference of less

than 2 from the final model were regarded as competitive models. Besides that, nonparametric one-way Kruskal–Wallis test followed by pairwise Wilcoxon rank-sum tests were performed to determine if ant abundance significantly differs across the four human activity levels investigated. All analyses were performed using JMP 15 (SAS Institute Inc., 2019).

RESULTS

Long-legged ant (*A. gracilipes*) distribution

Our field surveys showed that *A. gracilipes* distribution differed across the three sites across Penang Island. The highest occurrence of *A. gracilipes* was observed at the Bukit Jambul/Relau site, where *A. gracilipes* was recorded from all four sub-locations (Fig. 3). On the other hand, *A. gracilipes* was least frequently observed at the Youth Park site.

Within the Youth Park site, *A. gracilipes* was most abundant at the two sub-locations with high human activity, present on 45% of the baited index cards (Fig. 3). Furthermore, these two sub-locations were the only sub-locations in Youth Park where the highest abundance score of 5 was recorded. *A. gracilipes* was very uncommon at the sub-location with moderate human activity while being absent from the sub-location with low human activity.

Within the Sungai Ara site, *A. gracilipes* was most abundant at the sub-location with moderate human activity, occurring on 86% of the baited index cards and 16% of the index cards registering the highest abundance score of 5 (Fig. 3). Meanwhile, *A. gracilipes* was found in lower numbers at the sub-locations with low and high human activity and was never observed at the sub-location with very high human activity.

Within the Bukit Jambul/Relau site, the highest numbers of *A. gracilipes* were observed with low and moderate human activity (Fig. 3). *A. gracilipes* was found on slightly

more baited index cards at the sub-location with low human activity (87%) than at the sub-location with moderate human activity (84%). Still, only 11% of the index cards at the low human activity sub-location registered an abundance score of 5 compared to 16% at the sub-location with moderate human activity. Besides that, *A. gracilipes* was quite common at the sub-location with high human activity and least common at the sub-location with very high human activity, where *A. gracilipes* was only present on 4% of index cards.

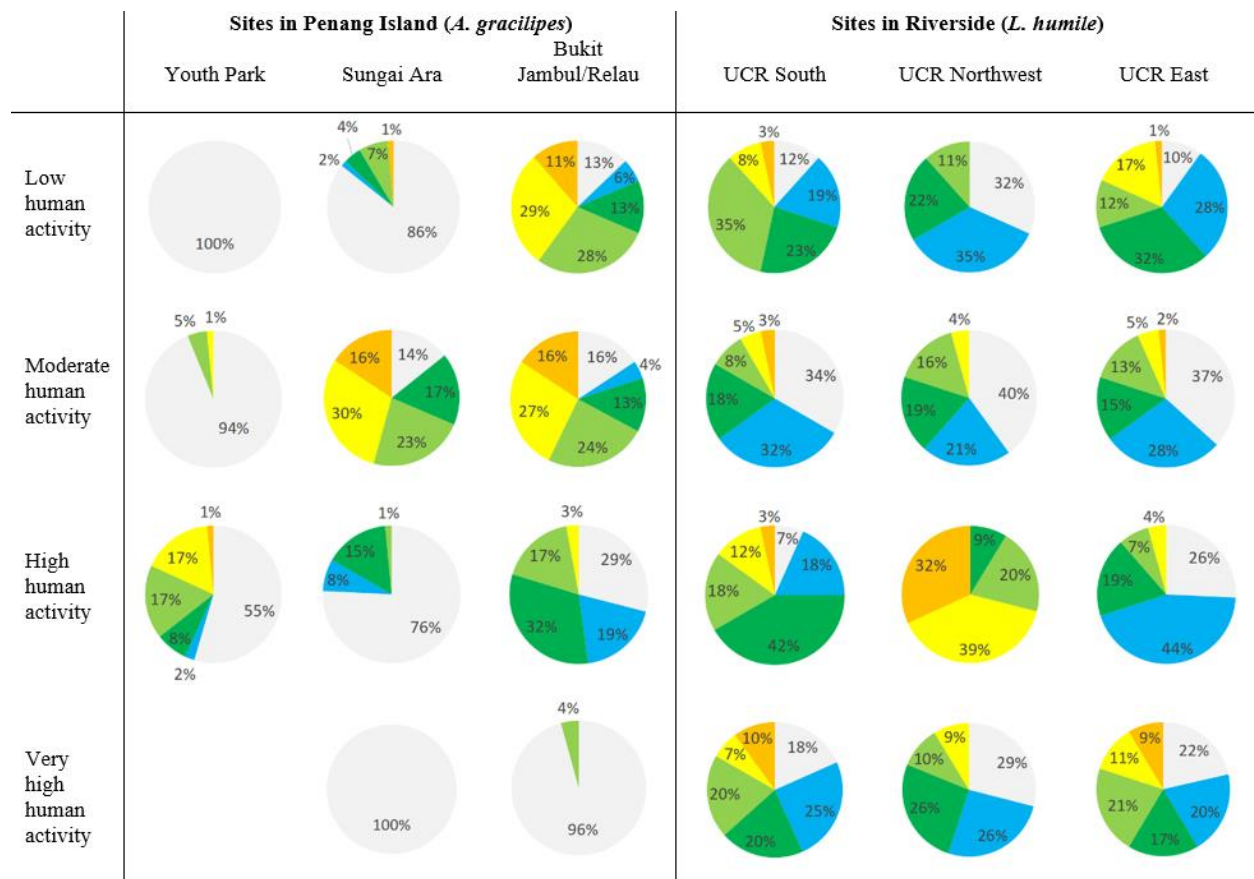


Figure 3. Percentage of baited index cards with different abundance scores of *A. gracilipes* and *L. humile* at the six study sites across four different levels of human activity (gray = abundance score 0; light blue = abundance score 1; dark green = abundance score 2; light green = abundance score 3; yellow = abundance score 4; orange = abundance score 5).

Table 1. Generalized linear model fitting results for average abundance score of *A. gracilipes* workers. Only the full model and models with Δ corrected Akaike's information criterion (AICc) <2 are presented.

Response variable	Independent variables	AICc	Δ AICc
Average abundance score of <i>A. gracilipes</i> workers	$\log(\text{no. of passersby} + 1) + [\log(\text{no. of passersby} + 1)]^2 + \text{temperature} + \text{relative humidity}$ (full model)	182.7	2.1
	$\log(\text{no. of passersby} + 1) + [\log(\text{no. of passersby} + 1)]^2 + \text{temperature}$ (final model)	180.6	0.0
	$\log(\text{no. of passersby} + 1) + [\log(\text{no. of passersby} + 1)]^2 + \text{relative humidity}$	180.7	0.1

Table 2. Parameter estimates for the most parsimonious (final) model of average abundance score of *A. gracilipes* workers.

Response variable	Independent variables	Estimate	SE	χ^2	<i>p</i> -value
Average abundance score of <i>A. gracilipes</i> workers	Intercept	-0.14	1.43	0.01	0.9204
	$\text{Log}(\text{no. of passersby}+1)$	1.25	0.35	16.62	<0.0001
	$[\text{Log}(\text{no. of passersby}+1)]^2$	-0.28	0.07	22.01	<0.0001
	Temperature	-0.02	0.05	0.10	0.7503

The generalized linear modeling fitting results indicated that the most parsimonious model included the linear and quadratic term of $\log(\text{no. of passersby} + 1)$ and temperature (Table 1). Based on the Δ AICc values calculated, the model including humidity instead of temperature was also highly qualified to explain the data (Table 1). In the final model, only the linear and quadratic terms of $\log(\text{no. of passersby})$ demonstrated a significant effect on the average ant abundance score ($P < 0.0001$, Table 2; Fig. 4a). The predicted average ant abundance score peaked when $\log(\text{no. of passersby} + 1)$ was 2.24 (= 8.39 passersby), corresponding to areas with moderate human activity (Fig. 4a). Further analysis with the Kruskal–Wallis test followed by pairwise Wilcoxon tests found that ant abundance was significantly lower in areas with very high human activity. Still, there was no significant difference in ant abundance between moderate

human activity and the other two levels of human activity ($\chi^2 = 27.81$, $d.f. = 3$, $P < 0.0001$; Fig 4b).

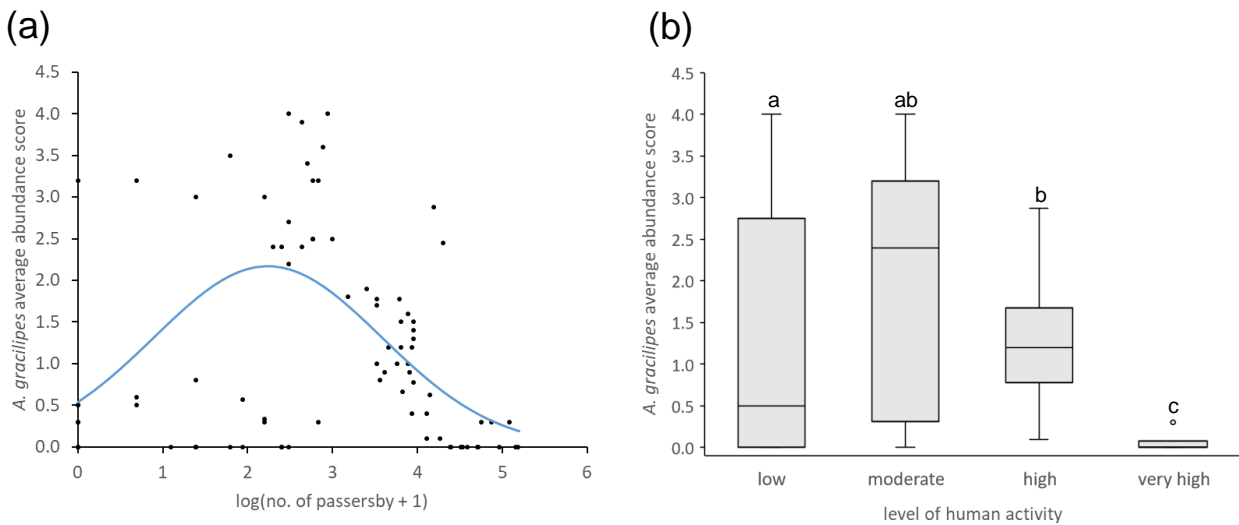


Figure 4. (a) Predicted average abundance score of *A. gracilipes* workers as a function of log(no. of passersby + 1) according to the final model. (b) Average abundance score of *A. gracilipes* workers recorded across the four different levels of human activity. Box plots labeled with different letters indicate significant differences ($P < 0.05$) between different levels of human activity (Wilcoxon rank-sum test).

Ant species composition of Penang sites

Thirty-four species of ants were collected from the three sites in Penang, comprising seventeen species from the subfamily Myrmicinae, eight species from the subfamily Formicinae, four species from the subfamily Dolichoderinae, four species from the subfamily Ponerinae, and one species from the subfamily Pseudomyrmicinae (Table 3). Aside from *A. gracilipes*, several species listed as major tramp ants by Wetterer (2015) were collected, including the tropical fire ant (*Solenopsis geminata* Fabricius), destroyer ant (*Trichomyrmex destructor* Jerdon), longhorn crazy ant (*Paratrechina longicornis* Latreille), robust crazy ant (*Nylanderia bourbonica* Forel) and ghost ant (*Tapinoma melanocephalum* Fabricius).

Table 3. List of ant species collected at the three Penang sites (SA = Sungai Ara, BJR = Bukit Jambul/Relau, YP = Youth Park), with “+” indicating the presence of a species at a particular sub-location.

Species	Very high human activity		High human activity				Moderate human activity			Low human activity		
	SA-A	BJR-A	YP-B	YP-C	SA-E	BJR-C	YP-A	SA-C	BJR-B	YP-D	SA-D	BJR-D
Subfamily Myrmicinae												
<i>Pheidole parva</i>	+		+	+	+	+	+	+	+		+	+
<i>Tetramorium</i> sp.	+	+	+	+	+	+		+	+			+
<i>Monomorium floricola</i>		+			+	+		+	+			
<i>Tetramorium bicarinatum</i>	+					+		+	+			
<i>Monomorium orientale</i>		+			+	+			+			
<i>Solenopsis geminata</i>		+				+		+				
<i>Trichomyrmex destructor</i>		+			+	+						
<i>Pheidole</i> sp. 1				+						+		
<i>Pheidole</i> sp. 2										+		
<i>Pheidole</i> sp. 3											+	
<i>Pheidole</i> sp. 4											+	
<i>Crematogaster</i> sp. 1								+				
<i>Crematogaster</i> sp. 2						+						
<i>Cardiocondyla</i> sp.						+						
<i>Carebara</i> sp.										+		
<i>Lophomyrmex</i> sp.	+											
<i>Proatta butтели</i>											+	
Subfamily Formicinae												
<i>Anoplolepis gracilipes</i>		+	+	+	+	+	+	+	+		+	+
<i>Paratrechina longicornis</i>	+	+	+	+	+	+	+	+	+			
<i>Nylanderia bourbonica</i>		+		+	+	+	+		+		+	
<i>Camponotus parius</i>	+					+			+		+	
<i>Nylanderia</i> sp.				+						+		

(Fig. 3). However, *L. humile* was most commonly observed at the high human activity sub-location and was present on 93% of index cards. The lowest numbers of *L. humile* were recorded at the sub-location with moderate human activity, with *L. humile* absent on 34% of index cards.

Within the UCR Northwest site, an extremely high abundance of *L. humile* was noted at the sub-location with high human activity, where *L. humile* was present on all deployed index cards, and 32% of index cards registered an abundance score of 5 (Fig. 3). *L. humile* was least common at the sub-locations with low and moderate human activity, as no index cards with abundance scores of 4 or 5 were observed at the former, while *L. humile* was absent on 40% of index cards at the latter.

Within the UCR East site, *L. humile* was most abundant at the sub-location with very high human activity, with 9% of index cards showing an abundance score of 5 (Fig 3). However, *L. humile* was most widespread at the low human activity sub-location and was found on 90% of index cards. In the two remaining sub-locations, *L. humile* was least abundant at the sub-location with high human activity. No index cards with abundance scores of 5 were recorded and least commonly found at the sub-location with moderate human activity as *L. humile* was absent on 37% of index cards.

The generalized linear modeling results indicated that the model with only temperature as the independent variable was the most parsimonious (Table 4). Still, the likelihood ratio test determined that this model did not differ significantly from the null model in predicting the average abundance score of *L. humile* workers ($\chi^2 = 0.6524$, $P = 0.4192$). None of the factors we considered in the modeling, including log(no. of passersby + 1), temperature, and relative humidity, demonstrated significant effects on the abundance of *L. humile*.

Table 4. Generalized linear model fitting results for average abundance score of *L. humile* workers. Only the full model and models with Δ corrected Akaike's information criterion (AICc) <2 are presented.

Response variable	Independent variables	AICc	Δ AICc
Average abundance score of <i>L. humile</i> workers	$\log(\text{no. of passersby} + 1) + [\log(\text{no. of passersby} + 1)]^2 + \text{temperature} + \text{relative humidity}$ (full model)	229.1	5.7
	None (final model)	224.6	1.2
	Temperature	223.4	0.0
	Relative humidity	224.0	0.6
	Temperature + relative humidity	224.6	1.2

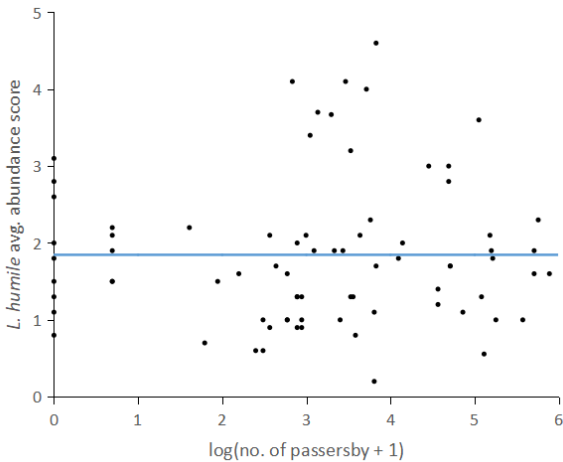


Figure 5. Average abundance score of *L. humile* as a function of $\log(\text{no. of passersby} + 1)$ indicated by the null model. The grand mean of the average abundance score was used to construct the best fit line.

Ant species composition at UCR

Only five species of ants were collected in total from the three sites in UCR, comprising three species from the subfamily Myrmicinae, one species from the subfamily Formicinae, and one species from the subfamily Dolichoderinae (Table 5). Apart from *L. humile*, two other alien species were recorded: the red imported fire ant (*Solenopsis invicta* Buren) and dark rover ant (*Brachymyrmex patagonicus* Mayr). The two remaining species were native: the thief ant (*Solenopsis molesta* Say) and *Monomorium ergatogyna* Wheeler. Surprisingly, the highest ant species diversity was found in the sub-locations with moderate human activity. The two native

species were also only encountered in sub-locations with moderate human activity. Elsewhere, *L. humile* appeared to be the dominant ant species encountered at all sub-locations and was often the only species present on the baited index cards.

Table 5. List of ant species collected at the three UCR sites (S = UCR South, NW = UCR Northwest, E = UCR East), with “+” indicating the presence of a species at a particular sub-location.

Species	Very high human activity			High human activity			Moderate human activity			Low human activity		
	UCR-S-A	UCR-NW-A	UCR-E-A	UCR-S-B	UCR-NW-B	UCR-E-B	UCR-S-C	UCR-NW-E	UCR-E-C	UCR-S-D	UCR-NW-D	UCR-E-D
Subfamily Myrmicinae												
<i>Solenopsis invicta</i>	+						+		+			
<i>Solenopsis molesta</i>								+				
<i>Monomorium ergatogyna</i>												+
Subfamily Formicinae												
<i>Brachymyrmex patagonicus</i>							+	+	+			+
Subfamily Dolichoderinae												
<i>Linepithema humile</i>	+	+	+	+	+	+	+	+	+	+	+	+

DISCUSSION

Our results show that human activity is a significant predictor of *A. gracilipes* distribution and abundance. Numerous studies have implicated human disturbance as an important factor in influencing the spread of tramp ants through several mechanisms. Human modification of the environment often creates more suitable microclimates conducive to the establishment of tramp ants, allowing otherwise unfavorable environments to be colonized (Tschinkel, 1988; Menke et al., 2007; Roura-Pascual et al., 2011). Among the sub-locations with low human activities we

surveyed, *A. gracilipes* was only absent from the sub-location in the Youth Park site, which was situated in a mostly undisturbed primary forest. In contrast, the other two sub-locations in Sungai Ara and Bukit Jambul/Relau had apparent signs of human disturbance, such as tarred roads and partially cleared vegetation. Field experiments have found that non-forest ant species such as *S. geminata* could not survive inside forested habitats due to microclimatic factors (Torres, 1984). The strong association of *A. gracilipes* with human-modified environments with reduced vegetation cover suggests that similar factors may inhibit its colonization of undisturbed forests.

Since *A. gracilipes* primarily utilizes budding for reproduction, they mainly depend on human activity for long-distance dispersal (Rao et al., 1991a; Haines et al., 1994). Consequently, human activity is correlated with increased propagule pressure, which increases the likelihood of tramp ant propagules being introduced and becoming established (Pyšek et al., 2010; Rizali et al., 2010). Besides that, urbanized environments can augment resource availability for urban ants. This can be in the form of nectar and honeydew-producing hemipterans due to irrigation (Menke & Holway, 2006) or in the form of human foods, which provide a novel resource for urban ants (Youngsteadt et al., 2015). These factors would favor the establishment of *A. gracilipes* in disturbed environments.

However, our data does not support a linear relationship between human activity and *A. gracilipes* abundance. The highest ant abundance was found at moderate levels of human activity, resembling patterns noted in the previous study in Taiwan (Lee et al., 2021). *A. gracilipes* abundance was lowest in areas with the highest levels of human activity in our study. The low abundance of *A. gracilipes* in highly urbanized areas could be due to increased interspecific competition with other species of urban ants (Vonshak & Gordon, 2015). In our surveys, the highest number of major tramp ant species was similarly collected from sites with

very high and high levels of human activity, suggesting that competition with other behaviorally dominant ant species could limit the population size of *A. gracilipes* in urban areas (Fig. 6). Previous lab experiments have shown that while *A. gracilipes* showed limited aggression towards and were occasionally even repelled by smaller urban ant species such as *Monomorium orientale*, *Monomorium floricola*, *Tapinoma* spp., and minor workers of *Pheidole parva*. They were more aggressive and were very successful in killing many of the larger urban ant species such as *Paratrechina longicornis*, *Trichomyrmex destructor*, *Solenopsis geminata*, and major workers of *Pheidole parva* (Chong and Lee, 2010). Nonetheless, even the aforementioned ant species, which normally lost to *A. gracilipes* under laboratory conditions, were still commonly collected from areas inhabited by *A. gracilipes*, indicating that they can still successfully compete with *A. gracilipes* in the field to some extent. Similar observations were made in cacao plantations where the presence of *A. gracilipes* did not affect the species richness of non-forest ants (Bos et al., 2008).

Aside from interspecific competition with other tramp ants, reduced availability of suitable nesting sites for *A. gracilipes* may impede the colonization of highly urbanized locations. Typically, *A. gracilipes* rarely constructs nests in soil and tends to nest in pre-existing spaces, most often in rocky crevices under boulders and foundations, but also under fallen vegetation and other miscellaneous debris (Fluker & Beardsley, 1970; Baker, 1976; Lewis et al., 1976; Haines & Haines, 1978; Rao & Veeresh, 1991b). In urbanized areas, the preferred nesting sites of *A. gracilipes* are less common due to the high proportion of concrete structures (Lee et al., 2021). Areas with very high levels of human activity are usually more well-maintained, resulting in a general absence of debris in most spaces, along with a reduction in the number of suitable crevices in man-made structures, which *A. gracilipes* can potentially nest in (personal

observation). In the presence of other tramp ants, *A. gracilipes* may only become dominant in certain areas with abundant nesting sites (Fluker & Beardsley, 1970).

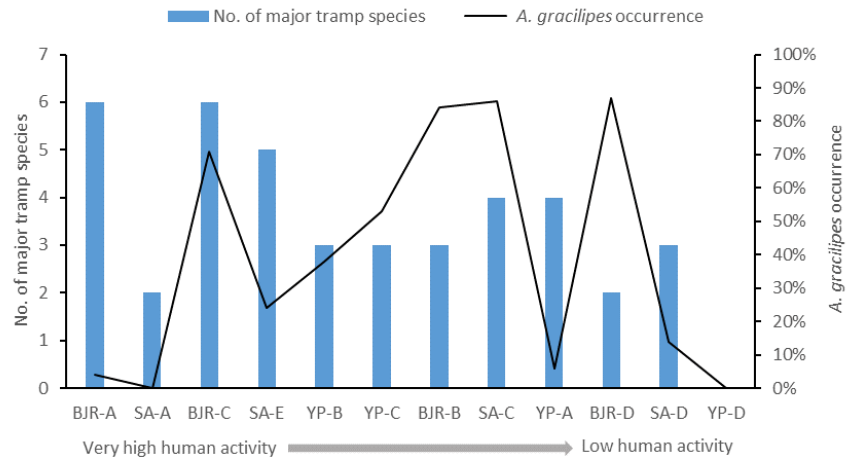


Figure 6. The number of major tramp ant species and occurrence of *A. gracilipes* on baited index cards at each of the sub-locations in Penang.

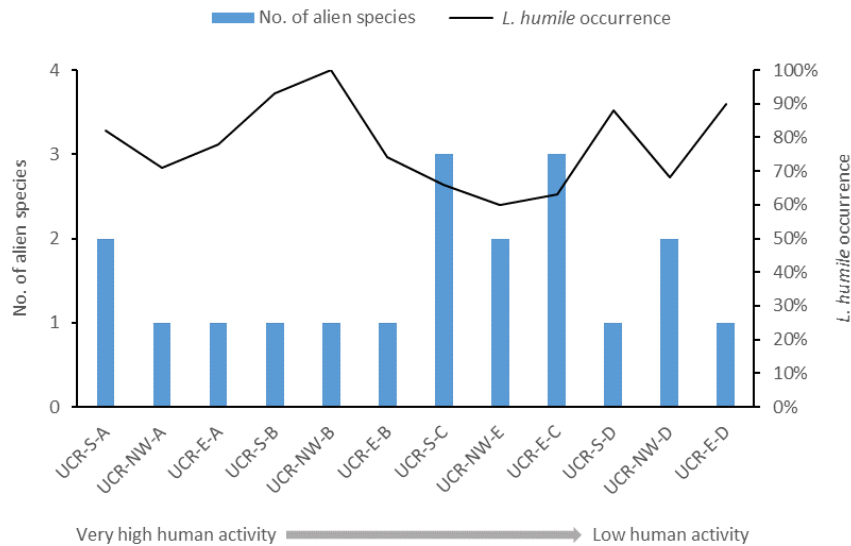


Figure 7. The number of alien ant species and occurrence of *L. humile* on baited index cards at each of the sub-locations in Riverside.

Compared to our *A. gracilipes* surveys which showed a clear trend between human activity and ant abundance, our surveys in Riverside did not find any association between human activity and *L. humile* abundance. This is surprising as numerous studies have consistently found

a strong link between *L. humile* distribution and human disturbance (Menke et al., 2007; Roura-Pascual et al., 2011; Vonshak & Gordon, 2015). The lack of observable trends between *L. humile* abundance and human activity in our data could be an artifact of the small geographical area covered by the survey. Aside from human activity, temperature and relative humidity were also found not to affect *L. humile* distribution. Instead, the distribution of *L. humile* may depend on other environmental variables not evaluated in our surveys, such as soil moisture (Menke & Holway, 2006; Menke et al., 2007).

Akin to our findings for *A. gracilipes*, evidence suggests that interspecific competition with other urban ant species could affect *L. humile* abundance. In areas where other alien ant species (*S. invicta* and *B. patagonicus*) were collected, *L. humile* on baited index cards was usually lower than in areas where *L. humile* was the only species present (Fig. 6). *S. invicta* and *L. humile* are known to compete strongly, resulting in mutual exclusion from habitats (LeBrun et al., 2007). Unexpectedly, alien ant species richness was highest in the sub-locations with moderate human activity. This contrasts with previous findings and the results of our Penang surveys, where urban ant species richness peaked in areas with the most human disturbance (Vonshak & Gordon, 2015).

In summary, the high abundance of *A. gracilipes* in areas of moderate human activity likely stems from a combination of suitable microclimatic conditions, high propagule pressure, increased resource availability, reduced interspecific competition with other urban species, and plentiful nesting sites. For *L. humile*, more studies will be necessary to further elucidate the effects of human activity. Still, our findings demonstrate that *L. humile* distribution may be limited by the presence of other competitive urban ants as well.

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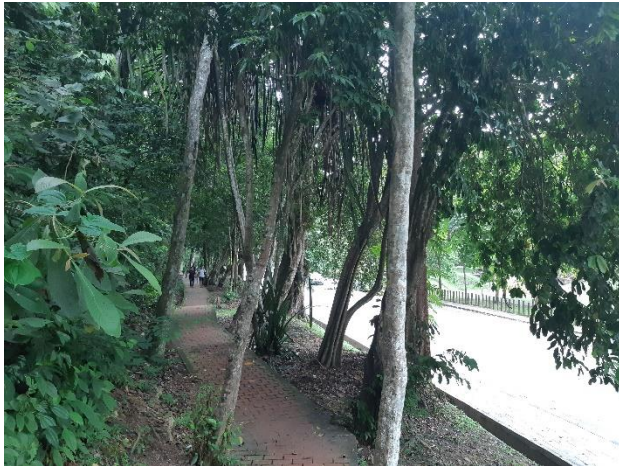
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SUPPLEMENTARY MATERIALS

Youth Park sub-locations



Top left: YP-A ($5^{\circ}26'6''$ N $100^{\circ}17'45''$ E)

Top right: YP-B ($5^{\circ}25'47''$ N $100^{\circ}17'47''$ E)

Bottom left: YP-C ($5^{\circ}26'7''$ N $100^{\circ}17'36''$ E)

Bottom right: YP-D ($5^{\circ}26'3''$ N $100^{\circ}17'30''$ E)

Sungai Ara sub-locations



Top left: SA-A ($5^{\circ}19'22''$ N $100^{\circ}16'11''$ E)

Top right: SA-C ($5^{\circ}18'15''$ N $100^{\circ}15'38''$ E)

Bottom left: SA-D ($5^{\circ}18'34''$ N $100^{\circ}15'21''$ E)

Bottom right: SA-E ($5^{\circ}18'60''$ N $100^{\circ}16'3''$ E)

Bukit Jambul/Relau sub-locations



Top left: BJR-A (5°19'51" N 100°16'47" E)

Top right: BJR-B (5°20'46" N 100°16'26" E)

Bottom left: BJR-C (5°20'25" N 100°16'27" E)

Bottom right: BJR-D (5°20'29" N 100°17'0" E)

UCR Northwest sub-locations



Top left: NW-A ($33^{\circ}58'33''$ N $117^{\circ}19'37''$ W)

Top right: NW-B ($33^{\circ}58'29''$ N $117^{\circ}19'58''$ W)

Bottom left: NW-D ($33^{\circ}58'35''$ N $117^{\circ}19'33''$ W)

Bottom right: NW-E ($33^{\circ}58'41''$ N $117^{\circ}19'58''$ W)

UCR South sub-locations



Top left: S-A (33°58'20" N 117°19'41" W)

Top right: S-B (33°58'16" N 117°19'36" W)

Bottom left: S-C (33°58'12" N 117°19'33" W)

Bottom right: S-D (33°58'7" N 117°19'34" W)

UCR East sub-locations



Top left: E-A ($33^{\circ}58'29''$ N $117^{\circ}19'26''$ W)

Top right: E-B ($33^{\circ}58'27''$ N $117^{\circ}19'16''$ W)

Bottom left: E-C ($33^{\circ}58'42''$ N $117^{\circ}19'16''$ W)

Bottom right: E-D ($33^{\circ}58'22''$ N $117^{\circ}19'19''$ W)