

Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*

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Summary

1. The number of individuals involved in an invasion event, or 'propagule size', has a strong theoretical basis for influencing invasion success. However, rarely has propagule size been experimentally manipulated to examine changes in invader behaviour, and propagule longevity and success.

2. We manipulated propagule size of the invasive Argentine ant *Linepithema humile* in laboratory and field studies. Laboratory experiments involved *L. humile* propagules containing two queens and 10, 100, 200 or 1000 workers. Propagules were introduced into arenas containing colonies of queens and 200 workers of the competing native ant *Monomorium antarcticum*. The effects of food availability were investigated via treatments of only one central resource, or 10 separated resources. Field studies used similar colony sizes of *L. humile*, which were introduced into novel environments near an invasion front.

3. In laboratory studies, small propagules of *L. humile* were quickly annihilated. Only the larger propagule size survived and killed the native ant colony in some replicates. Aggression was largely independent of food availability, but the behaviour of *L. humile* changed substantially with propagule size. In larger propagules, aggressive behaviour was significantly more frequent, while *L. humile* were much more likely to avoid conflict in smaller propagules.

4. In field studies, however, propagule size did not influence colony persistence. *Linepithema humile* colonies persisted for up to 2 months, even in small propagules of 10 workers. Factors such as temperature or competitor abundance had no effect, although some colonies were decimated by *M. antarcticum*.

5. *Synthesis and applications.* Although propagule size has been correlated with invasion success in a wide variety of taxa, our results indicate that it will have limited predictive power with species displaying behavioural plasticity. We recommend that aspects of animal behaviour be given much more consideration in attempts to model invasion success. Secondly, areas of high biodiversity are thought to offer biotic resistance to invasion via the abundance of predators and competitors. Invasive pests such as *L. humile* appear to modify their behaviour according to local conditions, and establishment was not related to resource availability. We cannot necessarily rely on high levels of native biodiversity to repel invasions.

Key-words: aggression, biological invasions, biotic resistance, interspecific competition, resource availability

Introduction

The probability of an invasive species successfully establishing in a new location is a function of both the recipient environment

and the intrinsic aspects of the invader. The invader must be able to tolerate the climate of the recipient environment and the diversity of species in the local community (Elton 1958). Of the intrinsic aspects, propagule pressure has a strong theoretical basis for determining the likelihood of establishment (Grevstad 1999; Colautti, Grigorovich & MacIsaac 2006). Propagule pressure is a composite measure of propagule size (the number of individuals initially released into a region

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where they are not native) and propagule number (the number of separate release events) (Lockwood, Cassey & Blackburn 2005). There is evidence that the larger the initial release size, the higher the chances of establishment (Drake & Lodge 2006). Large incipient populations have a higher chance of establishment because they have an increased ability to retrieve resources, find suitable habitats (Chapman & Bourke 2001) and overcome unfavourable conditions (Moller 1996). Small incipient populations may not reproduce, or may not withstand unfavourable environmental and biological conditions (Sakata & Katayama 2001). In social insects, propagule size can relate to the number of workers in a single colony, the number of queens being introduced within a single colony, or the number of colonies being introduced.

Here, we examined the role of propagule pressure related to worker numbers for the invasive Argentine ant *Linepithema humile* Mayr. Initially from South America (Tsutsui *et al.* 2001), it has invaded many regions of the world (Holway *et al.* 2002). Its current and predicted distribution, based on climatic factors, indicates likely invasion of temperate and sub-tropical zones (Hartley, Harris & Lester 2006). Where *L. humile* invade, they disrupt communities and reduce invertebrate abundance (Ward 1987; Human & Gordon 1997). *Linepithema humile* colonies in introduced ranges are often unicolonial, forming supercolonies with genetically similar workers lacking intraspecific aggression (Tsutsui *et al.* 2001; Corin *et al.* 2007). Unicoloniality allows a high density of workers to be maintained, allowing rapid recruitment of workers to resources (Holway 1998). However, although previous studies have examined the role of *L. humile* abundance in foraging success (e.g. Walters & MacKay 2005), whether numerical abundance influences the probability of *L. humile* establishment in their introduced ranges remains speculative. Further, although behaviour probably plays an important role in invasion success (Holway & Suarez 1999) and it is known that invaders like *L. humile* can modify behavioural aspects such as foraging according to group size (Gordon 1995), little research has examined behavioural plasticity associated with propagule size and attributes of the recipient community.

According to the biotic resistance hypothesis, resistance can arise in areas with high biodiversity (Elton 1958) or from strongly interacting resident competitors independent of diversity (Baltz & Moyle 1993). Therefore, behavioural interactions between exotic and resident species over resources and space may even prevent an invasive species from becoming established in a community (Tilman 1999). In Australasia, when *L. humile* invades, it will have to compete and interact with native ants such as those in the genus *Monomorium*. The presence of venomous alkaloids (Don & Jones 1993) and a sting (Hölldobler & Wilson 1990) in many species of *Monomorium* are probably responsible for their competitiveness with invasive ants. For example, in Australia, *M. rothsteini* Forel resisted the more aggressive *Iridomyrmex* species (Andersen, Blum & Jones 1991). In an experiment testing the competitive mechanisms of *L. humile* against native ant species in California, *M. ergatogyna* Wheeler was seldom

displaced (Holway 1999). In New Zealand, *Monomorium antarcticum* (Smith) is the most common and widespread native ant (Brown 1958).

We used a combination of laboratory and field studies to examine the effect of propagule size (the number of worker ants) on incipient *L. humile* colony survival. In the laboratory, we examined the hypothesis that increasing propagule size would allow for an increased time of invader population persistence in the presence of a native competitor. In these laboratory experiments, we examined whether an increase in food would also increase propagule persistence, and whether propagule size and food abundance influences interspecific behaviour. To test these hypotheses, we used a similar experimental design to those of Wilson (1976) and Walters & MacKay (2005). Such laboratory experiments are not entirely representative of the natural environment, but allow an examination of behavioural interactions under controlled conditions. We further tested the role of propagule size on invader persistence in field trials by introducing *L. humile* colonies into novel, previously uninvaded territory.

Materials and methods

LABORATORY COLONIES

Linepithema humile were collected from Hastings (39°63' S, 176°85' E, and 39°63' S, 176°86' E) and Wellington (41°28' S, 174°76' E) and then combined in the laboratory as they are genetically similar and display no aggression towards each other (Corin *et al.* 2007). *Monomorium antarcticum* were collected from Wellington (41°28' S, 174°71' E) in July 2006. Colonies were placed in separate plastic containers (11 × 7 × 6 cm) with the sides painted with flulon™ (Polytetrafluoroethylene) to prevent escape. Each container had two 10-ml nesting tubes one-third filled with water, plugged with cotton wool, and covered with aluminium foil (Hee *et al.* 2000). They were fed daily with a mixed diet of 25% sugar water (via absorbent cotton wool), tuna in oil and peanut butter. Incubation temperature was 25 °C and humidity 50% with a light–dark cycle of 12:12 h light:dark.

PROPAGULE SURVIVAL AND GROWTH IN LABORATORY ARENAS

To examine *L. humile* propagule size establishment success in the presence of *M. antarcticum*, we used a factorial design with four categories of *L. humile* propagule size and two food treatments (low food and high food). The low food treatment had one food dish and the high food treatment had 10 food dishes. Food in these dishes consisted of approximately 0.3 g of scrambled egg, 0.6 g of peanut butter, 25% sugar water (via absorbent cotton wool), and one dead fly *Lucilia sericata* Meigen daily. Food items were mixed into individual shallow dishes (0.7 cm wall and 2.8 cm in diameter) and haphazardly placed inside the foraging arena. *Linepithema humile* propagules consisted of colonies of 10, 100, 200, or 1000 workers, each with two queens. Colonies were placed in individual nesting containers (14 × 9 × 8 cm) with the top half of all inner walls painted with flulon. Each nesting container had two nesting tubes. A plastic exit tube (5 cm long, 0.3 cm internal diameter) was inserted into one of the shorter container sides. Each *L. humile* propagule size (except for controls) was introduced into a foraging arena with an existing nest container

of 200 workers and two queens of *M. antarcticum*. The foraging arena was a plastic tray (72 × 34 × 13 cm) covered with fine white sand, with the inner walls painted with fluon. The nest containers of each species were placed at opposite ends of the foraging arena.

We introduced *M. antarcticum* into the foraging arena 2 days prior to introducing *L. humile* to allow *M. antarcticum* to lay claim to the arena. Both species were then monitored at least twice daily. The time until one of the ant species became extinct was recorded. If however, *L. humile* or both species persisted, we continued to supply food for up to 2 months. After 2 months, we considered the colonies to be established and likely to persist longer, and ended the trials by counting the total number of brood, workers and queens. Prior to statistical analysis, we examined all the data below for heteroscedasticity using Levene's test, and for fit to a normal distribution using the Shapiro–Wilk normality test. The data on propagule survival times did not conform to a normal distribution (Shapiro–Wilk $P < 0.001$). Therefore, we used two-way univariate Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2001). We performed the analysis on untransformed data without standardization, with the Bray–Curtis dissimilarity as the distance measure and 9999 permutations. The explanatory variables were propagule size and food amount; the response variable was survival time.

Some of the 1000 *L. humile* colonies persisted for the study duration. To determine sublethal effects of *M. antarcticum* presence on *L. humile*, a control treatment was included. Controls consisted of 1000 workers and two queens of *L. humile* in both food treatments, but with no *M. antarcticum*. Five replicates were used for each colony size per food treatment, except for the colonies of 1000 workers and controls which had three replicates. A two-way ANOVA tested for sublethal effects of *M. antarcticum* presence on the total number of *L. humile* workers alive at the end of 2 months and to compare the total number of brood (eggs, larvae and pupa) produced. Analysis was performed on untransformed data, as there was no evidence of heteroscedasticity (Levene's $P \geq 0.259$) or non-normality (Shapiro–Wilk $P \geq 0.428$).

INTERSPECIFIC INTERACTIONS IN LABORATORY ARENAS

Aggressive behaviour between *L. humile* and *M. antarcticum* was determined by observing both species for 120 s every 10 min for 3 h after introducing *L. humile* into arenas. The behavioural categories followed Rowles & O'Dowd (2007). Category 'ignore' (= 0) included contacts where no interest or aggression was displayed. If interest was shown via antennation, it was considered 'touch' (= 1). Contact where both ants retreated from each other quickly was scored as 'avoid' (= 2). Where contact included lunging, biting or leg-pulling it was regarded as 'aggression' (= 3). Prolonged (> 5 s) incidences of aggression, individuals locked together and active flexing of gasters in the use of stings or chemical defences, was scored as 'fighting' (= 4). Individual pairs of heterospecific ants were haphazardly chosen for observation as they moved within ~1 cm of each other. The species that moved toward the other species and initiated behavioural interactions was recorded. We gave a score to each species in cases where both species initiated contact at the same time. The behavioural data were analyzed using multinomial logistic regression. Individual scores from all replicate arenas were pooled in each treatment. The five categorical outcomes were the aggression ratings (0–4), which were modelled as being dependent on species (*L. humile* or *M. antarcticum*), low and high food availability (1 food tray per arena or 10 trays per arena), and propagule size (two queens and 10, 100, 200 or 1000 workers). The reference category for the analysis was *M. antarcticum*,

high food availability and a propagule size of 1000 workers. Only the main effects were included in the model. A full factorial model was undertaken, and although it fitted the data better (Cox and Snell's pseudo $R^2 = 0.273$), singularities in the Hessian matrix were encountered and P values and parameter estimates could not be calculated. Ordinal logistic regression procedures were also undertaken, and although results were similar, the fit was much poorer (Cox and Snell's pseudo $R^2 = 0.061$) than the presented analysis.

TESTS OF PROPAGULE SURVIVAL IN THE FIELD

The study was conducted in forest patches at Korokoro (Lower Hutt, New Zealand) (41°220' S, 174°870' E) in mid-summer to early autumn 2007. The study area consisted of closed forest patches on hills. On the extreme west side of the site, there was a single *L. humile* colony at the base of a pedestrian overpass. This colony appeared to be spreading from a larger infestation into new territory at a rate of 2–5 m per month during the 3 months prior to this study. We placed the nearest *L. humile* experimental colony 15 m away and the farthest one 130 m away from this single colony, keeping the experimental *L. humile* colonies as near as possible to the invasion front in case of accidental release. Experimental colonies were separated by ≥ 20 m.

We introduced *L. humile* propagules consisting of 10, 100 or 1000 worker treatments, each with one queen. Each propagule size (colony size) was placed in a clear plastic nest container measuring 14 × 6 × 4 cm. Each container had two nest tubes. There were five 1 mm exit holes on shorter sides of the container 1–2 cm up from the base. Preliminary trials demonstrated that the exit holes were sufficient for workers to exit, but not queens. Several 0.5 mm holes under the flanges of the containers were made for ventilation. Fluon was painted over the upper holes and upper inner walls to prevent these being used as exit holes.

After placing *L. humile* in the containers, we sealed the exit holes and left the colonies in an incubator for 5 days (with food consisting of 25% sugar water and tuna in oil) to acclimatize. After 5 days, we took the colonies to the field and allowed the ants to exit the containers and forage. Propagule treatments of 100 and 1000 *L. humile* had six replicates, while due to limiting numbers of queen ants, the propagule size of 10 workers had only four replicates. We also used control colonies consisting of 10, 100 and 1000 workers and one queen per worker category with two replicates each. The control containers had no exit holes, and the ants were fed 25% sugar water, tuna in oil and a single fly (*L. sericata*) every 3–4 days. These controls tested the influence of abiotic conditions on *L. humile*. All colonies were checked every 3–4 days to monitor worker presence in the nest boxes and the survival time of the queen (thus, all queens had died at the end of the experiment).

Small temperature data loggers (Onset, Pocasset Massachusetts) were placed in all containers to record the temperature every 30 min and calculate a daily mean of minimum, maximum and mean temperatures for each container. We used these daily mean temperatures to calculate an overall mean of minimum, maximum and mean temperatures for the entire 3 months of this study. A Kruskal–Wallis analysis was used to test for differences in average daily mean temperatures between controls and experimental containers. Pitfall traps (7 cm top diameter × 9 cm deep) were used to sample relative resident ant abundance prior to introducing *L. humile* and at the study conclusion. Two pitfall traps were haphazardly placed 20–25 cm from each container. Pitfall traps were one-third filled with preservative made from equal parts water and monoethylene glycol with a few drops of detergent to break the surface tension. Traps were removed after 5 days and all ants were identified and counted.

A one-way ANCOVA was used to test for the effect of propagule size on colony survival time. Only after log transformation did the data conform to a normal distribution (Shapiro–Wilk $P \geq 0.625$). A Levene's test revealed homogeneity of variance after the log transformation for both variables ($P \geq 0.566$). The covariates were the total abundance of two common resident ant species [*M. antarcticum* and *Pachycondyla castanea* (Mayr)] sampled from pitfall traps.

Results

PROPAGULE SURVIVAL AND GROWTH IN LABORATORY ARENAS

For the colony survival analysis, we compared only the 10, 100 and 200 *L. humile* colony treatments, as all the 1000 worker replicates persisted for ≥ 2 months. PERMANOVA test revealed propagule size to have a significant effect on *L. humile* colony survival ($P = 0.037$, Fig. 1, see Supplementary Material Table S1 for the full PERMANOVA table). Post hoc analysis revealed that a propagule size of 10 workers had a shorter survival time than that of 200 workers ($P = 0.008$). However, the mean survival time for a propagule size of 10 workers was not statistically different from a propagule size of 100 workers ($P = 0.304$), and that of a propagule size of 100 workers was not different from that of 200 workers ($P = 0.170$). The effect of food treatment was not statistically significant ($P = 0.125$, Fig. 1). There was no significant interaction between food and propagule size treatments ($P = 0.682$).

In the treatments with 1000 *L. humile* workers, entire *L. humile* colonies were never annihilated by *M. antarcticum*. Instead, *L. humile* colonies persisted for 61 days (~ 1464 h) in all replicates for both food treatments. Three of the six *M. antarcticum* colonies were annihilated by *L. humile* during

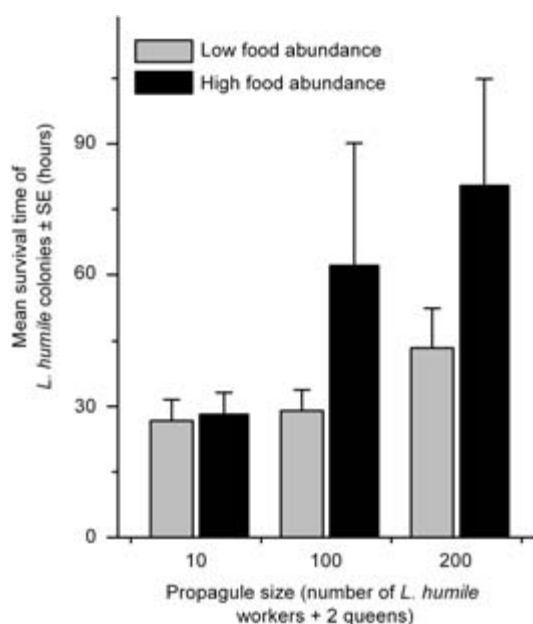


Fig. 1. Mean survival time (\pm SE) of *L. humile* laboratory colonies in the presence of *M. antarcticum* in low food and high food treatments.

this time (two colonies in the high food treatment which persisted for 21 and 31 days, and one colony in the low food treatment which persisted for 25 days). In replicates where both ant species persisted, each species appeared to take turns in completely dominating all food resources on the arena floor lasting for up to several days. The species not foraging would entirely retreat into their nest boxes, defending entrances. Two-way ANOVA indicated that the presence of *M. antarcticum* in the 1000 *L. humile* treatments significantly increased the mortality of *L. humile* workers ($F_{1,8} = 31.416$, $P < 0.001$). The mean number of *L. humile* workers alive in control colonies (655 ± 42 , means \pm SE) was twice that of experimental colonies (313 ± 48) where *M. antarcticum* were present. We speculate that the $\sim 35\%$ mortality in the control treatment with *L. humile* alone represented dietary inadequacies, as the diet we provided may not have been equally nutritious for long-term population growth of both species. Increasing food availability did not have a significant effect on the survival of *L. humile* workers (two-way ANOVA $F_{1,8} = 2.456$, $P = 0.156$). Nor was the interaction term between the absence of *M. antarcticum* and the amount of food significant for *L. humile* worker survival (two-way ANOVA $F_{1,8} = 0.733$, $P = 0.417$). The mean number of brood in experimental colonies (569 ± 47) was higher than control colonies (390 ± 93), although not significantly so (two-way ANOVA $F_{1,8} = 2.568$, $P = 0.148$). Nor did the amount of food (two-way ANOVA $F_{1,8} = 0.688$, $P = 0.431$) or the interaction between these two variables (two-way ANOVA $F_{1,8} = 0.019$, $P = 0.895$) have a significant effect on brood production by *L. humile*.

INTERSPECIFIC INTERACTIONS IN LABORATORY ARENAS

Linepithema humile propagules of 10, 100 and 200 workers were not able to defend their nests against *M. antarcticum* which entered nests and killed the *L. humile* workers and queens. During *M. antarcticum* raids, *L. humile* queens and several workers hid between spaces in the nesting tubes and aluminium foil or vacated their containers and sought refuge in the foraging arena or on top of their nesting boxes. *Linepithema humile* queens always attempted escape in the company of several attending *L. humile* workers (Fig. 2a). At a colony size of 1000 workers, *L. humile* resisted invasion and attacked workers of *M. antarcticum* at the *M. antarcticum* nests entrances. *Monomorium antarcticum* workers blocked the *M. antarcticum* nest exit tubes in defence by sitting in an acrobatic manner with gasters and heads pointing towards the attackers, behaviours similar to those which we observed at our field site (Fig. 2b). Despite such aggressive behaviour, *L. humile* continued to attack *M. antarcticum* at their exit tubes by biting and pulling the limbs of workers (Fig. 2c). Once exit tubes were cleared, *L. humile* entered the *M. antarcticum* nests. In contrast to *L. humile*, *M. antarcticum* queens attempted to escape alone which appeared to increase the chance of being killed by *L. humile* workers (Fig. 2d).

In all observed interactions, more than one *L. humile* worker was needed to kill individual *M. antarcticum*.

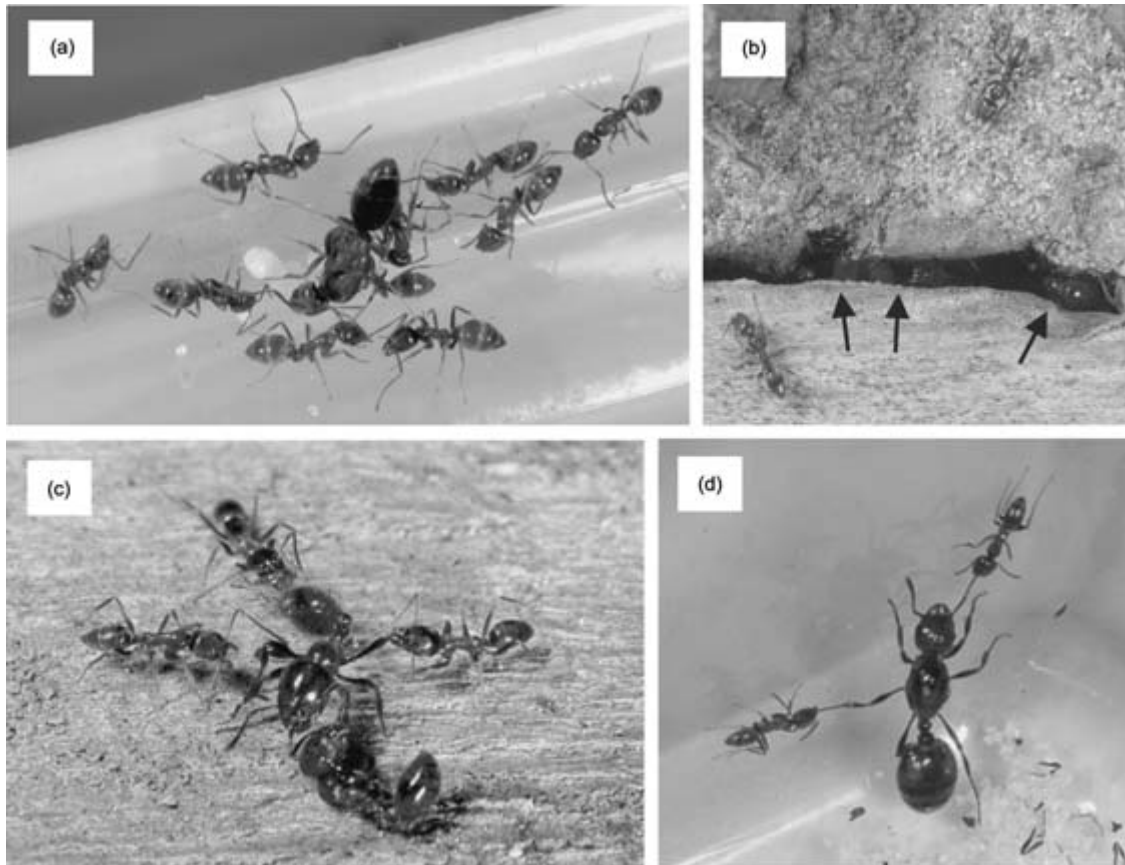


Fig. 2. Interactions between *L. humile* and *M. antarcticum*. (a) A *L. humile* queen in the company of several workers during an escape after colony invasion by *M. antarcticum*. (b) *Linepithema humile* attacking *M. antarcticum* workers that have blocked their nest entrance (shown by arrows) at the field site. (c) A *M. antarcticum* worker being attacked by three *L. humile* workers. Note that the ant lying in the forefront is an *M. antarcticum* worker that has had all of its limbs amputated by *L. humile*. (d) A lone *M. antarcticum* queen being attacked by two *L. humile* workers in the corner of the foraging arena. (Photographs by P.J.L.)

Linepithema humile killed their opponents through joint effort by one or more workers pulling on a limb while another amputated the *M. antarcticum* appendage. The opponent was then left to die (Fig. 2c). In contrast, *M. antarcticum* workers always attacked singly, killing *L. humile* in one-on-one encounters. *Monomorium antarcticum* appeared to use stings and noxious chemicals against *L. humile*.

The likelihood ratio test from the logistic regression showed significant effects of species ($\chi^2 = 351.04$, d.f. = 4, $P < 0.001$), food availability ($\chi^2 = 11.75$, d.f. = 4, $P = 0.019$), and propagule size ($\chi^2 = 147.73$, d.f. = 12, $P < 0.001$). *Linepithema humile* displayed a high frequency of interaction in all behavioural categories (Fig. 3). Conversely, *M. antarcticum* displayed fewer benign interactions and showed high frequencies of 'aggression' and 'fighting'. Increasing propagule size substantially decreased the frequency of benign interactions and increased the frequency of aggressive behaviours (Fig. 3a). For *M. antarcticum*, increasing *L. humile* propagule size led to a less pronounced change in behaviour, with a slight increase in the frequency of benign interactions but still largely aggressive behaviour (Fig. 3b). The model produced a relatively poor fit to the full data set (Cox and Snell's pseudo $R^2 = 0.155$), which was primarily due to a low classification

success in the low aggression categories. Supplementary Material Table S2 shows the nominal parameter estimates from the model, in which the response of each factor was examined for each behavioural category in comparison with a reference treatment. For example, *L. humile* was found to be significantly more likely to display a behavioural category of 0 than *M. antarcticum* (odds ratio = 2.776, $P < 0.001$). Similarly, *L. humile* was significantly more likely to display behaviours in categories 1 and 2. *Linepithema humile* only exhibited less behavioural aggression than *M. antarcticum* in aggression category 3 (odds ratio = 0.624, $P < 0.001$; Supplementary Material Table S2).

For *L. humile*, the frequency of 'aggression' and 'fighting' was high in low food treatments and only slightly decreased with high food (Fig. 3c). However, there was no difference between low and high food treatments for 'ignore' and 'touch' behaviours. This effect was observed in the multinomial logistic regression wherein the only significant factor associated with food was observed under behavioural category 2, indicating that ants were significantly less likely to display aggression in high than in low food treatments (odds ratio = 0.654, $P = 0.001$). In all other behavioural category comparisons, there were no significant differences in aggression displayed

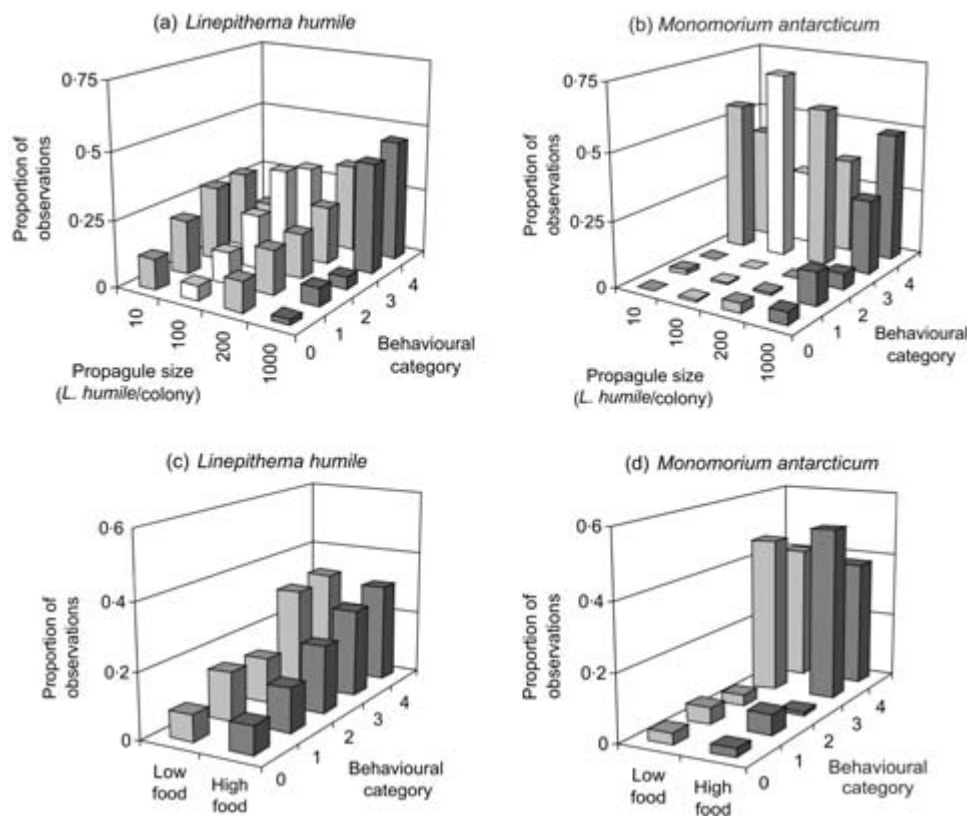


Fig. 3. The effect of species, propagule size and amount of food on behavioural interactions. Propagule sizes were 10, 100, 200 and 1000 *L. humile* workers and two queens per worker category. Ant behavioural categories were 'ignore' (0), 'touch' (1), 'avoid' (2), 'aggression' (3) and 'fighting' (4). The amounts of food were low food (one food dish per foraging arena) and high food (10 food dishes per foraging arena).

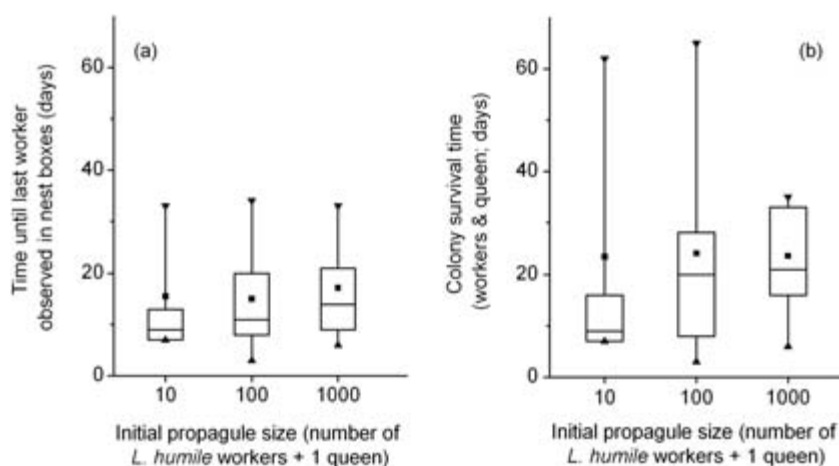


Fig. 4. Survival times for the *L. humile* field propagules. (a) The time until the last *L. humile* worker was observed in the nest boxes, and (b) the time until colony death (queen with workers). The bars show 50% of the data with 25th and 75th percentiles; whiskers show minimum and maximum data values; the lines across the bars is the median; mean is shown by filled squares.

between the food treatments ($P \geq 0.504$, Supplementary Material Table S2). Similarly to results examining propagule size, *M. antarcticum* was highly aggressive irrespective of food availability (Fig. 3d).

TESTS OF PROPAGULE SURVIVAL IN THE FIELD

Monomorium antarcticum entered *L. humile* colonies and exterminated some colonies within 3 days of introduction. We also observed *L. humile* workers in three colonies of 1000 workers fighting with *M. antarcticum* outside the nest

containers. Some 100 and 1000-worker colonies established foraging trails 24 h after introduction. However, these trails gradually disappeared over three to four consecutive days. Four epigeic resident ant species were observed at the field site: *Discothyrea antarctica* Emery, *M. antarcticum*, *P. castanea*, and *Strumigenys perplexa* (Smith). Only eight *D. antarctica* and five *S. perplexa* were observed and were never observed to interact with *L. humile*, which were therefore not included as covariates in the ANCOVA. The two other species *M. antarcticum* and *P. castanea* were more abundant and occurred in 41% (13 of 32) and 56% (18 of 32) of the pitfall traps, respectively.

There was no statistically significant effect of propagule size on the survival times of *L. humile* colonies when examining for the presence of workers only (one-way ANCOVA $F_{2,11} = 0.208$, $P = 0.816$, Fig. 4a) or for queens and workers (one-way ANCOVA $F_{2,11} = 0.062$, $P = 0.940$, Fig. 4b). The average survival times of queens was greater than the mean time until the last worker was observed in all the colony sizes, with queens living for up to 31 days in the absence of workers. No significant effects of the covariates *M. antarcticum* or *P. castanea* were observed in either ANCOVA model ($P \geq 0.389$).

Some variations in temperature were observed between propagule placement sites in the mean daily average (range = 15.5 to 20.2 °C), the mean daily minimum (13.8 to 16.2 °C), and the mean daily maximum temperatures (17.2 to 27.4 °C). However, no significant differences in any of the temperature variables were observed between treatments (Kruskal–Wallis $P \geq 0.059$). The mean temperature of nest containers was marginally but significantly higher in experimental nest boxes (16.99 ± 0.31 °C; mean \pm SE, $N = 16$) compared to controls (15.69 ± 0.45 °C; $N = 6$) (Mann–Whitney- $U = 21$, $P = 0.049$). In control colonies, 100% worker mortality was observed for the colony size of 10 workers. After 37 days and 65 days, queens in two colonies of the 10-worker replicates also died. Workers of the 100 and 1000 control treatments survived until we ended the experiment, but with a mean worker mortality of 66.5% and 67.9%, respectively. These mortality rates in controls were high, but similar or higher rates of mortality have been observed with *L. humile* even when well-fed and in controlled laboratory environments for a similar period (Grover *et al.* 2007).

Discussion

Our laboratory findings support the general theory that larger propagule sizes have a higher chance of survival and establishment (Blackburn & Duncan 2001; Drake & Lodge 2006). Our field experiments, in contrast, found no significant relationship between propagule size and colony survival for the range of worker numbers studied. *Linepithema humile* could survive the abiotic conditions of the area, as evidenced by the control treatments. Food may have been limiting at the field site. However, replicates of the small propagules survived as long as our largest propagule treatment. Our initial hypothesis was that the survival of small propagules would be related to variation in *M. antarcticum* densities at the field site. Although the ANCOVA found no significant effects, we did observe *M. antarcticum* to destroy *L. humile* field colonies. The *L. humile* behavioural plasticity observed in the laboratory probably complicates any simple relationship between propagule survival and native ant abundance. In the laboratory, *L. humile* altered its response to competitor abundance and showed adaptive escape behaviours, probably extending propagule survival times under field conditions. Thus, propagule size may not necessarily be a good predictor of persistence times under field conditions.

Interestingly, in the treatments with the largest *L. humile* propagule size, we observed co-existence of these two ant species in the laboratory environment for > 2 months. Each

species took turns dominating the food resources and then retreated to their nest boxes. Although *M. antarcticum* substantially reduced *L. humile* numbers, *L. humile*'s ability to raise brood appeared unaffected. A large propagule size has the numerical advantage of allowing some members of the colony to engage in interspecific interactions while others maintained normal colony functions. In the presence of very aggressive resident ant species like *M. antarcticum*, only large incipient colonies could resist and have sufficient workers to maintain normal colony function (Holway 1999; Walters & Mackay 2005). In the field environment, we did not observe the co-existence of these species, either from naturally occurring nests or experimentally placed colonies. However, the experimental field colonies of *L. humile* were all eventually killed. A relatively specific abundance ratio of each species is probably required for their persistence for any substantial time period. *Linepithema humile* generally maintains a uniclonal lifestyle in their invaded range (Holway *et al.* 2002). A key advantage of uniclonality is that there are huge numbers of workers for colony defence or attack. It is thus unlikely that co-existence will occur in the field environment. Elsewhere, few other ant species co-exist with *L. humile* (e.g. Ward 1987).

ROLE OF COMPETITION FOR RESOURCES

Competition for resources and space is an important factor structuring ant communities (e.g. Hölldobler & Wilson 1990; Sanders & Gordon 2003). In the laboratory study, *L. humile* survived only slightly longer in high relative to low food treatments. However, this effect was not statistically significant, indicating that the availability of food had no or little role in *L. humile* propagule survival. Aggression in *L. humile* has previously been considered unrelated to resources such as food or nest sites (Holway 1999; Zee & Holway 2006). The observed nest raiding of *L. humile* on *M. antarcticum* probably reflects the species' intolerance for co-occurring epigeic ants (DeKock & Giliomee 1989).

Interference and exploitative competition has been studied extensively in *L. humile*. Most studies have found that through sheer numbers of recruits, *L. humile* is successful at both forms of competition thereby breaking 'dominance–discovery' trade-off (or the trade-off in life-history patterns between the abilities to find and to control resources) (e.g. Holway 1999). However, their success in interference competition may be simply a by-product of their aggressive nature. At high densities, *L. humile* are more likely to display aggression toward heterospecific ants, in contrast to other ant species (Human & Gordon 1999; Zee & Holway 2006). Even in the complete absence of resources, *L. humile* behaves aggressively towards other ants (Zee & Holway 2006). Studies exploring behavioural interactions of *L. humile* with other ant species have shown that *L. humile*'s competitive interference ability is related to numerical abundance (Human & Gordon 1996; Holway 1999; Rowles & O'Dowd 2007). But the large numbers required for this success are probably related to the methods used to kill other ants rather than direct competition for resources. In order to kill workers of other ant species, *L. humile* requires

a group or pack attack (Fig. 2c). Thus, a large colony size enhances the ability of *L. humile* to kill other ant colonies. As a potentially adaptive consequence of such aggressive behaviour, limiting resources become available.

BEHAVIOURAL PLASTICITY AND PROPAGULE SIZE

Propagule size had a much more substantial effect on *L. humile* behaviour in laboratory experiments than resources moderating levels of aggression. *Linepithema humile* appeared to be able to determine its own colony size, or relative competitor abundance, and modify the aggressive response. An ability of ants to assess competitor abundance and then modify their behaviour has been previously observed (e.g. Wilson 1976), although not previously associated with invasion success.

At small colony sizes, *L. humile* showed high frequencies of 'ignore', 'touch' and 'avoid' behaviours suggesting an avoidance or escape strategy, and they were unable to defend their nest against *M. antarcticum*. They often vacated their nests and attempted to climb over the walls of the foraging arenas. In such circumstances, *L. humile* queens were always accompanied by workers. Such behaviours may allow small incipient *L. humile* colonies to escape and survive in a natural environment. If small colonies do survive, they may increase their numbers quickly, as observed in other laboratory studies (Hee *et al.* 2000).

In their native environment, *L. humile* and other invasive species like the fire ant *Solenopsis invicta* occupy an intermediate competitive position wherein they are extirpated from resources by more dominant ants, but also dominate other species (LeBrun *et al.* 2007). They may have evolved behaviour associated with both assessing competitor abundance and determining when to fight or flee. When fleeing, they show considerable adaptive behaviour with groups of attending workers exiting with queens after colony invasion. Dominant ants may be less able to modify their behaviour, as we observed with *M. antarcticum*. *Monomorium* produce venom which is of high repellency towards other species (Andersen *et al.* 1991; Holway 1999), and they can be very aggressive. Brightwell (2002) similarly observed high levels of aggression by *M. antarcticum* irrespective of *L. humile* densities; native ants were generally highly aggressive irrespective of competitor density. Similarly, LeBreton *et al.* (2007) demonstrated non-adaptive responses of the dominant native New Caledonian ants to the invasive little fire ant *Wasmannia auropunctata*. The type of behavioural plasticity exhibited by *L. humile* probably facilitates invasion success and may explain why ant species are over-represented in the list of 'world's worst invasive alien species' (Lowe, Browne & Boudjelas 2000). It is possible that the majority of ant species on this list occupy an intermediate competitive position in their native range, thus having a considerable degree of behavioural plasticity. An absence of behavioural plasticity perhaps makes an ant community more at risk from an invasion event, or an individual species less likely to succeed as a biological invader.

Attempts to predict invasion success have had limited success for most organisms (Hulme 2006) including ants (Lester 2005). Increasing numbers of studies invoke propagule

pressure as a (or the) fundamental driver of invasions (Richardson & Pyšek 2008). Our results indicate that propagule size may have limited predictive power for species displaying behavioural plasticity. In one of the few studies incorporating behavioural plasticity as a predictive factor in exotic bird establishment, enhanced predictions of invasion success were obtained (Sol, Timmermans & Lefebvre 2002). Behavioural aspects are seldom incorporated into models to predict invasion success and may be hidden from observation without detailed experiments on each potential invasive species. Echoing Holway & Suarez (1999), we recommend that aspects of animal behaviour be given much more consideration in attempts to model invasion success. Without detailed knowledge of the behavioural adaptations that may enhance invader success, an emphasis on managing invasion pathways may be more efficient (Hulme 2006). Areas of high biodiversity are thought to offer biotic resistance to invasion via the abundance of predators and competitors. For example, a diverse invertebrate community on a rocky seashore may efficiently utilize space resources and exclude an invasive species (Stachowicz, Whitlatch & Osman 1999). However, invasive pests such as *L. humile* appear to modify their behaviour according to local conditions and their establishment may not be related to resource availability. Perhaps the failure to find evidence for biotic resistance elsewhere may be related to behavioural plasticity of invaders in response to local communities. We cannot necessarily rely on high levels of native biodiversity to repel invasions, nor can managers assume that small propagules are unlikely to establish and thrive in a new environment. Conversely, workers in areas of translocations for conservation purposes or practitioners of biological control may not need large introduction propagules if the species of interest displays similar behavioural plasticity.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Full PERMANOVA table from the analysis of propagule size and food effects on *L. humile* survival in laboratory studies

Table S2. Results from the multinomial logistic regression, modelling ant behaviour as a function of species, food abundance, and propagule size

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