Resource competition assays between the African big-headed ant, *Pheidole megacephala* (Fabricius) and the invasive Argentine ant, *Linepithema humile* (Mayr): mechanisms of inter-specific displacement

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Abstract. 1. The spread of Argentine ants, *Linepithema humile* (Mayr), in introduced areas is mainly through the displacement of native ant species owing to high inter-specific competition. In South Africa, *L. humile* has not established in the climatically suitable eastern and northern escarpments dominated by the African big headed ant, *Pheidole megacephala* (Fabricius), probably owing to local biotic resistance.

2. Inter-specific aggression, at the individual and colony level, and competition for a shared resource were evaluated in the laboratory.

3. Aggression between the two ant species was very high in all of the assays. Both species suffered similar mortality rates during one-on-one aggression assays, however, during symmetrical group confrontations, *L. humile* workers showed significantly higher mortality rates than *P. megacephala* workers. During asymmetrical group confrontations both species killed more of the other ant species when they had numeric advantage. Both ant species located the shared resource at the same time; however, once *P. megacephala* discovered the bait, they displaced *L. humile* from the bait through high inter-specific aggression, thereafter dominating the bait for the remainder of the trial.

4. The results demonstrate the potential of *P. megacephala* to prevent the establishment and survival of incipient *L. humile* colonies through enhanced resource competition and high inter-specific aggression. This is the first study to indicate potential biotic resistance to the spread of *L. humile* in South Africa.

Key words. Biotic resistance, foraging efficiency, inter-specific competition, *Linepithema humile*, *Pheidole megacephala*.

Introduction

Invasive species often arrive as small propagules that must survive a wide range of abiotic and biotic barriers in the recipient environment to successfully colonise, establish, persist, spread, and naturalise (Richardson *et al.,* 2000; Chapman & Bourke, 2001; Walters & Mackay, 2005). Suitable abiotic and biotic conditions define niche space and influence invasibility of an environment by colonising species (Richardson *et al.,* 2000; Le Breton *et al.,* 2005; Von Holle & Simberloff, 2005; Menke *et al.,* 2007). At each of these phases of the invasion process biotic factors such as propagule pressure and

biotic interactions with biota already present in the recipient environment, determines the ability of the introduced species to successfully establish; whereas abiotic factors such as environmental suitability further affect the ability of the incipient populations of the introduced species to persist and spread (Moller, 1996; Shea & Chesson, 2002; Krushelnycky *et al.,* 2005; Hartley *et al.,* 2010).

Invasive ants commonly excel at both resource exploitation (Davidson, 1998), locating and retrieving resources faster than other ant species (Morrison, 1996; Le Brun *et al.,* 2007) and interference competition, through their ability to usurp resources from native ants and displace them, particularly those who share similar ecological requirements (Heterick *et al.,* 2000; Holway *et al.,* 2002a; Lach, 2005; Le Breton *et al.,* 2007). Colony level attributes such as worker size and number positively influence the proficiency to exploit available resources or effectively

defend them from other ant species (Nowbahari *et al.,* 1999). This influences the ability of different ant species to co-exist within a community (Davidson, 1998; Le Brun & Feener, 2007). Although invasive ants commonly reduce the abundance and species richness of native ant communities (Holway, 1999; Carpintero *et al.,* 2007), recipient communities with ecologically dominant ant species may limit the spread of aggressive invasive ants through biotic resistance (Elton, 1958; Hoffmann *et al.,* 1999; Walters & Mackay, 2005; Wetterer *et al.,* 2006; Blight *et al.,* 2010).

The Argentine ant, *Linepithema humile* (Mayr), is one of the best studied invasive ants (Holway *et al.,* 2002a; Wetterer *et al.,* 2009; Vogel *et al.,* 2010). Originally native to Argentina and parts of Brazil (Suarez *et al.,* 2001; Holway *et al.,* 2002a; Wetterer *et al.,* 2009), it now occupies a global distribution as a direct result of human movements (Suarez *et al.,* 2001; Sunamura *et al.,* 2009; Vogel *et al.,* 2010; van Wilgenburg *et al.,* 2010), often occupying these human-influenced environments which offer a suitable niche opportunity (Holway *et al.,* 2002b; King & Tschinkel, 2006). *Linepithema humile* is successful globally owing to its highly aggressive behaviour and large colony sizes which allow this ant to rapidly colonise, establish, and spread in new environments (Sunamura *et al.,* 2009). In South Africa, *L. humile* was first recorded in the late 1800s in Stellenbosch (Skaife, 1955; Prins *et al.,* 1990), Western Cape, which is characterised by a Mediterranean climate similar to its native range in Argentina, but has since established in both urban and natural areas in six of the nine provinces, not all characterised by a Mediterranean climate (Luruli, 2007). However, it has not established successfully in the eastern and northern parts of the country, dominated by the African big-headed ant, *Pheidole megacephala* (Fabricius) (Prins *et al.,* 1990; Majer & de Kock, 1992; Luruli, 2007), even though these regions are climatically suitable for *L. humile* (Roura-Pascual *et al.,* 2004; Luruli, 2007; Roura-Pascual *et al.,* 2011). Thus, local biotic resistance has been proposed to explain the current distribution of *L. humile* in South Africa (Luruli, 2007).

Pheidole megacephala is an endemic to Africa, originating from tropical regions of sub-Saharan Africa (Wilson & Taylor, 1967; Ross & Trager, 1990; Bolton, 1995; Dejean *et al.,* 2005; Moreau, 2008; Fournier *et al.,* 2012; Wetterer, 2012). Although not well studied (Holway *et al.,* 2002a), *P. megacephala* has been introduced in many parts of the world (Haskins & Haskins, 1965; Heterick, 1997; Hoffmann *et al.,* 1999; Heterick *et al.,* 2000; Lach, 2005; Dejean *et al.,* 2008; Fournier *et al.,* 2009; Wetterer, 2012), where it has been shown to be aggressive to both native and invasive ants such as *L. humile* (Krushelnycky *et al.,* 2005) and *Anoplolepis gracillipes* (Smith) (Kirschenbaum & Grace, 2008) found within its territory (Fluker & Beardsley, 1970; Jones *et al.,* 2001; Wetterer & Wetterer, 2004; Kirschenbaum & Grace, 2008). Originally it was suggested that the mutually exclusive distribution pattern of *L. humile* and *P. megacephala* was influenced by climate and altitude, with *L. humile* showing a preference for the higher, cooler altitudes (Cole *et al.,* 1992; Krushelnycky *et al.,* 2005). More recent studies however have included biotic factors to further explain these distribution patterns (Kirschenbaum & Grace, 2007, 2008; Lach, 2008). From aggression bioassays, Kirschenbaum and Grace (2008)

suggested that *P. megacephala* and *L. humile* maintain their mutually exclusive distribution through high levels of aggression, whereas Lach (2008) showed that they shared temporal foraging patterns and resources which brought them further into conflict and also contributed to their disparate distributions on tropical islands where they have both been introduced (Jones *et al.,* 2001; Krushelnycky *et al.,* 2005).

There is a paucity of empirical studies investigating the factors that facilitate successful range expansion in invasive ant species. Many studies have focussed on the influence of abiotic factors on range expansion of invasive ants (Roura-Pascual *et al.,* 2004; Menke & Holway, 2006, Menke *et al.,* 2007; Roura-Pascual *et al.,* 2011), with few studies investigating the importance of biotic factors in limiting or facilitating the spread of *L. humile* (Walters, 2006). Many of these studies are largely based on bait sampling and generalisations with regards to biological characteristics of invasive ants as measures to predict invasion success (Holway, 1999; Richardson *et al.,* 2000; Holway *et al.,* 2002b). However, detailed studies on the direct interactions between native ants and invasive ants are wanting, and are needed to understand how invasive ant species colonise new environments and which of their biological traits are likely to enhance their invasion success (Blight *et al.,* 2010; Vonshak *et al.,* 2012). For *L. humile*, the combination between abiotic conditions and inter-specific competition from native ant species may limit the extent to which these ants invade new environments as small propagules, largely because niche availability and suitability is determined by abiotic factors as well as the presence of competitive dominant ant species (Holway, 1999; Thomas & Holway, 2005; Walters & Mackay, 2005). Therefore, the aim of this study was to evaluate the inter-specific competition between *L. humile* and *P. megacephala* using behavioural assays that evaluated individual and colony level aggression as well as competition for shared resources. This study uses laboratory experiments to test the hypothesis that biotic resistance from *P. megacephala* is a plausible explanation for the current distribution patterns of *L. humile* in South Africa.

Materials and methods

Ant colonies and laboratory maintenance

The distribution of the African big-headed ant, *P. megacephala*, and the invasive Argentine ant, *L. humile*, in South Africa are mutually exclusive (Fig. 1). We conducted the study in the laboratory and staged interactions between these two species that would normally occur under field conditions. Eighteen colonies of *P. megacephala* were collected [identification of all colonies collected was confirmed using the recently published key from Wetterer (2012)] along the eastern and northern escarpment of South Africa along its known distribution in the Kwazulu-Natal and Limpopo Provinces; and 18 nests of *L. humile*, were collected from Stellenbosch and Jonkershoek in the Western Cape Province, South Africa (Fig. 1). Intra-specific aggression was high between colonies of *P. megacephala*, with no aggression found between nestmates during control aggression assays (see Roulston *et al.,* 2003; Mothapo & Wossler 2011
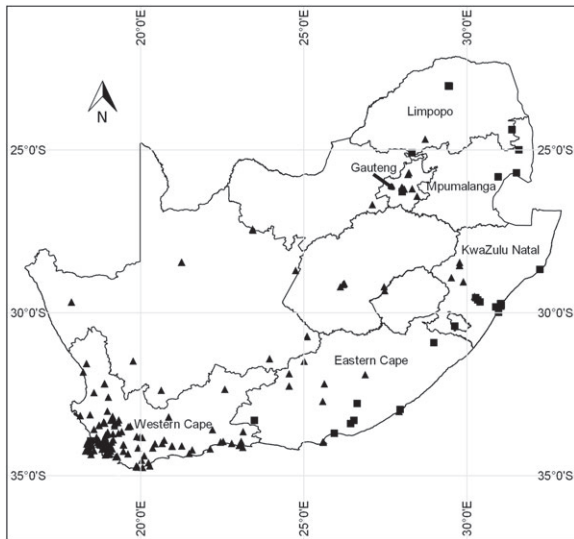
Fig. 1. Distribution of *Linepithema humile* (▲) and *Pheidole mega-cephala* (■) in South Africa, showing the main provinces where the two ant species are found.

for similar experiments) indicating multicoloniality (N. Moth-apo, pers. obs.). *Linepithema humile* forms two behaviourally, chemically, and genetically distinct supercolonies with the large supercolony distributed throughout a large part of the country and the smaller colony limited to the Agulhas region of the West-ern Cape (Lado, 2008; Mothapo & Wossler, 2011). Ants were collected in a wide range of habitats including urban areas and in nature reserves. We located nests by laying small baits, con-sisting of cat food, directly on the ground and followed ants returning to the nests having fed on the bait. We also located the nests by overturning small rocks and looked for small soft mounds of soil which often were the nest entrances in the case of *P. megacephala*. Whole nests (consisting of workers, brood, and queens) were dug out, using gardening trowels, and trans-ferred into 4.5-litre plastic containers lined with Fluon™ (Flu-oropolymer Dispersion; Whitford plastics Ltd, Runcorn, U.K.) on the sides to prevent ant escape and transported to the labora-tory. All colonies were maintained under laboratory conditions at $25 \pm 2\,°C$, 40% RH and a LD 12:12 h cycle. The nest con-tainers had a small amount of soil which was misted with water twice weekly to maintain soil moisture levels optimal for ants. The ants were provided with a diet of 0.25 M sugar water daily, water *ad libitum* and were fed pin-head crickets, *Acheta domes-ticus* (Linnaeus), twice weekly when experiments were not in progress.

Aggression bioassays

We conducted recognised one-on-one aggression assays, sym-metrical, and asymmetrical group confrontations modified from Buczkowski and Bennett (2008) and Blight *et al.* (2010) to assess the fighting potential of both these species. Inter-specific competition over a shared resource using laboratory colonies of these two ant species was also assessed (see Resource

competition assay). Owing to the differences in head size rel-ative to their body, *P. megacephala* majors were not included in the aggression bioassays. Moreover, based on field observations, *P. megacephala* major workers only came out when nests were disturbed and were rarely found on foraging trails, also body size between *P. megacephala* majors (∼3 mm) (Wetterer, 2012) and *L. humile* (∼2.6 mm) workers (Wild, 2004) were dif-ferent, emphasising the use of only *P. megacephala* minors (∼2–2.6 mm) (Wetterer, 2012) during one-on-one and group assays. For the one-on-one aggression assays, two randomly selected workers from each colony of *P. megacephala* ($n = 18$ colonies) and *L. humile* ($n = 18$ nests) were paired in an 8-ml glass pill vial lined with Fluon™ 1 cm from the bottom. Ten individuals were paired per colony, and the ants were observed for 2 min. A maximum score, based on the most intense level of aggression recorded within the 2 min, was recorded for each trial. For the symmetrical group interactions, 20 workers per colony/nest were randomly selected ($n = 17$ colonies of *P. mega-cephala* and $n = 17$ nests of *L. humile*) and paired together in a Fluon™-lined glass Petri dish ($10 \times 4\,cm^2$). Similarly, for the asymmetrical group confrontations, 20 *P. megacephala* workers were paired against 10 *L. humile* workers ($n = 12$ colonies/nests per species) and 10 *P. megacephala* workers were paired against 20 *L. humile* workers ($n = 12$ colonies/nests per species) in a Fluon™-lined glass Petri dish ($10 \times 4\,cm^2$). The workers of each species were collected using an aspirator and transferred into an individual Fluon™-lined glass pill vial (8 ml). Both groups of ants were allowed to settle for 2 min, after which both ant groups were simultaneously transferred to the glass Petri dish. The ants were observed for 5 min and behavioural interactions were recorded at 1, 3 and 5 min. The behavioural interactions between the two workers were scored according to the meth-ods of Suarez *et al.* (1999) where 1 – antennate with no aggres-sive response, 2 – avoidance (on contact, ants stay on oppo-site ends of vial), 3 – aggression (lunging, biting, pulling) and 4 – fighting (prolonged aggression or fight). The number of aggressive (categories 3 and 4) and non-aggressive (1 and 2) interactions observed during each trial (5 min) were counted. At the end of each trial we recorded the number of dead ants of each species. Nestmates were paired during one-on-one and group confrontations to validate the experimental approach. No aggression occurred between nest/colony members.

Resource competition assay

We assessed the ability of these two ant species to compete for resources in a laboratory controlled experiment. We first assessed the ability of each species to exploit and assimilate a resource in the absence of a competitor (Baseline), then the potential of each species to interfere with the other during resource competition, pairing equally sized *L. humile* (nests) and *P. megacephala* colonies. We used the three measures associated with foraging success in ants: Discovery time – time taken to discover a resource; Recruitment intensity – the number of nestmates recruited to a resource over a given time period; and Retrieval – the amount of resource removed by ants within a given time (Hölldobler & Wilson, 1990; Davidson, 1998; Morrison, 2000). The data for retrieval were not used in the

analyses during interaction experiments as we did not mark or quantify the amount of food each species removed, only their presence on and around the bait.

Experiments were conducted in plastic arenas ($120 \times 60 \times 15$ cm^3) lined with Fluon^{TM} to prevent ant escape. The floor of the arena was lined with Plaster of Paris which kept an even surface, and was regularly moistened to prevent dust and maintain a moist surface for ants to walk on. We established 15 experimental colonies, from original stock colonies, consisting of 500 workers (which included ~30 majors for *P. megacephala* – represents the proportion of majors found in intact colonies), one queen and several brood pieces. Ants were starved for 48–72 h prior to resource exploitation trials to maintain an equal state of hunger and ensure that the ants were sufficiently hungry to start foraging during the experiment (Buczkowski & Bennett, 2008; Blight *et al.,* 2010; Mathieson *et al.,* 2012). We used bait consisting of a mixture of 3 : 1 : 2 parts tuna, honey, and water. This bait is widely used in ant baiting studies and represents a clumped resource that is too large for an individual ant to move and requires the recruitment of colony members (Holway, 1999; Human & Gordon, 1999; Le Brun *et al.,* 2007; Luruli, 2007) to remove it efficiently.

Twenty four hours prior to the start of the baseline experiment ($n = 15$ colonies each of *L. humile* and *P. megacephala*), a nest container was placed against the edge of the arena, opened, and a bridge made of cardbox was attached to the top of the container and the arena floor allowing the ants to move freely between the arena and nestbox. The ants were allowed to roam the arena to acclimatise. For the interaction experiment ($n = 17$ colonies each of *L. humile* and *P. megacephala*), the starved experimental colonies of both species were placed on opposite ends of the arena and the ants were not allowed to roam to prevent potential fighting. The bait was placed 60 cm from the nesting box in the centre of the arena. A circle with a 5 cm radius was measured around the bait location point and this was used as the observation area for all interactions. During the interaction experiment, behavioural interactions occurring between the two species within the given circumference around the resource were recorded. Scan sampling was used to randomly record the interactions between any interacting pair of ants within a given period. Every 2 min for 10 min at the 10, 20, 30, 60 and 90 min time interval of each trial a 10 s scan was done. The interactions were scored in the same way as described for the individual and group aggression bioassays. The number of aggressive (category 3 and 4) and non-aggressive (category 1 and 2) encounters were counted for the 90-min trial. At the end of each trial, the numbers of dead ants within the given circumference of the bait were counted, as a result of the aggressive interactions that occurred around the resource.

One gramme of the bait was weighed using a microbalance (Explorer-OHAUS with a weighing range of 0.001–410 g) and placed on a 2×2 cm^2 weighing paper. Making sure that no ants were present within 30 cm of the bait location, the bait was placed on the demarcated point (60 cm from nesting box) within the circle centre. A second bait was placed alongside the arena to measure weight loss by desiccation. The three measures associated with foraging success, discovery time, recruitment intensity, and the amount of resource retrieved, were recorded once the bait was placed in the demarcated area. Recruitment intensity was recorded by counting the number of ants present within the circle circumference around the bait for 1 min every 10 min for a total of 90 min, after which the trial was ended. The experimental bait and the control bait were weighed to measure the amount of food retrieved by the ants and corrected for water loss during the baseline experiment.

Statistical analysis

The proportion of aggressive interactions was calculated from the total number of interactions for all the trials; only the proportion of aggressive interactions is presented. At the end of each trial in the one-on-one assay, mortality was recorded for each species. A score of 1 was given when a worker of either species was dead and 0 when the worker was still alive with no injury. A McNemar's test for paired dichotomous categorical data with continuity correction was used to compare the mortality of *L. humile* and *P. capensis* during the one-on-one assays. Worker mortality of each ant species during the group interactions were compared using either a Wilcoxon-signed ranks test or a Paired-samples *t*-test.

A Wilcoxon-signed ranks test or a Mann–Whitney *U*-test was used to compare the time taken to discover the resource for each species during the assays. The recruitment intensity, number of ants at the bait per 10 min interval, was averaged across all the trials for the two species and recruitment curves were generated. The amount of resource consumed by each ant species was assessed and compared using a Mann–Whitney *U*-test for independent samples. Worker mortality of each ant species was compared using a Paired-samples *t*-test. To determine whether there was an effect of competitor presence on foraging efficiency, recruitment pattern (recruitment effort over time) and intensity without a competitor was compared with the recruitment effort and intensity when competing with a rival for a shared resource, for each of the two species using two-way repeated measures ANOVA with Greenhouse–Geisser correction because sphericity was violated. Statistical significance was accepted at $P < 0.05$ and all analyses were conducted in SPSS 20.0 statistical software (SPSS Inc., Chicago, Illinois).

Results

Aggression bioassays

During one-on-one interactions, 83% of all interactions were aggressive (Fig. 2a) with a maximum score of 4 during most of the interactions resulting in a 52% mortality rate for *P. megacephala* (94 dead individuals from 180 trials) compared with a 46% mortality rate for *L. humile* (83 dead individuals from 180) (Fig. 2a); however, this was not statistically significant (McNemar's $\chi^2 = 0.83$, $n = 180$, $P > 0.05$). Aggression between the two ant species during symmetrical group interactions was high (94%, Fig. 2b), with a significantly higher mortality of *L. humile* compared with *P. megacephala* ($Z = -1.99$, $P \leq 0.05$, Fig. 2b). The higher mortality of *L. humile* workers during symmetrical
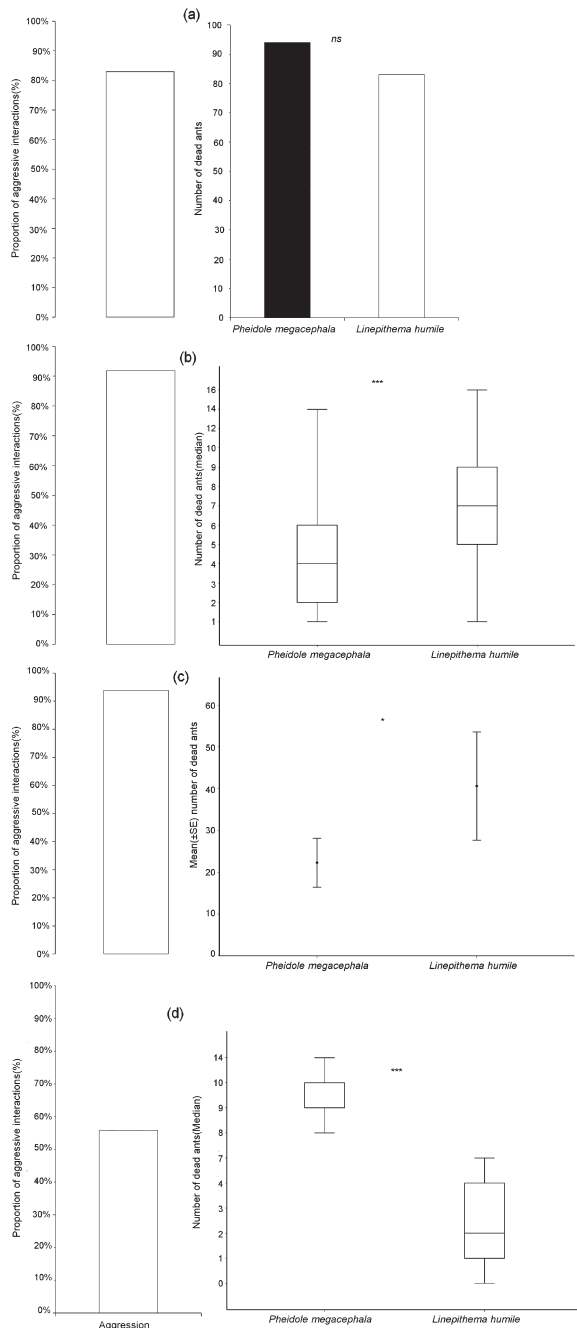
group interactions suggests that *P. megacephala* dominated the fights, being more lethal than *L. humile*. Interactions remained highly aggressive between these two ant species in asymmetrical group interactions (Fig. 2c,d), with both *P. megacephala* and *L. humile* showing superior fighting ability when they had numeric advantage as evident in the mortality rates. High levels of aggression (92%) were observed when *L. humile* had numeric advantage and resulted in significantly high levels of mortality for *P. megacephala* [$t_{(11)} = -8.01$, $P < 0.001$, Fig. 2c]. When *P. megacephala* had a numeric advantage, the level of aggression was only 56% yet *L. humile* still suffered high losses ($Z = -2.94$, $P < 0.001$, Fig. 2d). Both species employed both physical and chemical aggression.

Resource competition

There was no significant difference in the time taken to discover food between *L. humile* (203.3 ± 76.70) and *P. megacephala* (216.4 ± 82.1) in the absence of a competitor ($U = 70.5$, $Z = -1.74$, $n = 30$, $P > 0.05$, Fig. 3a). Both species also showed similar levels of recruitment intensity (Fig. 3b) and retrieved similar amounts of the bait ($U = 93$, $Z = -0.81$, $n = 30$, $P > 0.05$, Fig. 3c). During the resource competition experiment, both species rapidly moved from the nest into the arena, and were equally fast in discovering the bait ($Z = -0.91$, $P > 0.05$; Fig. 4a). Having discovered the bait, *L. humile* rapidly recruited in high numbers within the first 20 min of the assay, thereafter, however, the number of *P. megacephala* workers recruited increased with a concomitant decline in *L. humile* numbers, suggesting that *L. humile* workers were displaced from the bait by *P. megacephala* (Fig. 4b). The recruitment effort changed significantly across time for both *L. humile* [$F_{(2.94, 82.19)} = 4.39$, $\epsilon = 0.37$, $P = 0.007$] and *P. megacephala* [$F_{(2.92, 81.93)} = 16.02$, $\epsilon = 0.37$, $P < 0.001$]. However, *L. humile* recruited significantly less workers when having to compete for a resource with *P. megacephala* [$F_{(1)} = 17.77$, $P < 0.001$], whereas *P. megacephala*'s worker numbers were unaffected by *L. humile* presence [$F_{(1)} = 2.33$, $P = 0.14$]. The high levels of aggression observed during the interactions of these two ant species (Fig. 4c), as well as the high mortality of *L. humile* (40.6 ± 6.1) in comparison to *P. megacephala* (22.3 ± 2.8) [$t_{(16)} = 4.46$, $P < 0.001$, Fig. 4d], suggests that *P. megacephala* outcompeted *L. humile* at the bait through very high levels of aggression.

Discussion

Both *L. humile* and *P. megacephala* are intrinsically aggressive and were highly antagonistic to each other from individual through to colony level interactions. High levels of aggression were evident during one-on-one bioassays which are contrary to previous studies which have suggested that aggression bioassays are context dependent and consequently one-on-one aggression bioassays do not reflect accurate aggression levels between species, contending that fights between two ant species were more likely to occur between groups of ants rather than between individual foraging workers (Roulston *et al.*, 2003; Buczkowski
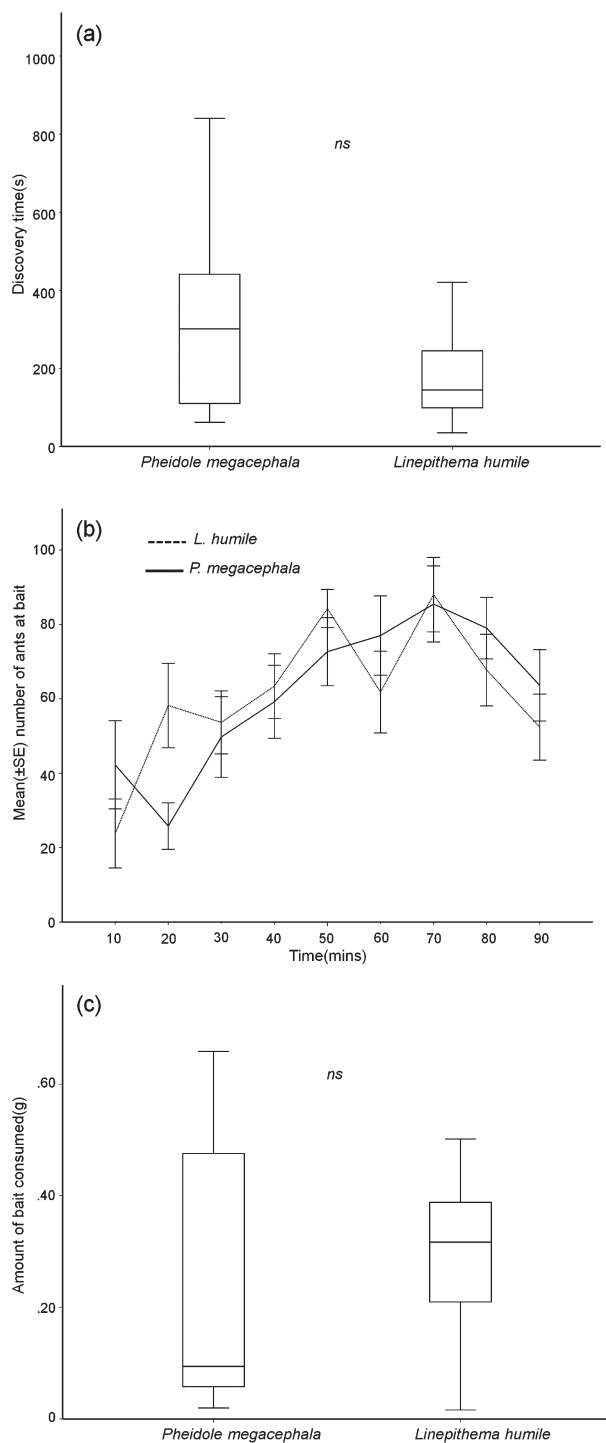

Fig. 2. Proportion of aggression and mortality rates per trial of *Linepithema humile* and *Pheidole megacephala* during (a) one-on-one interactions ($n = 180$ trials), McNemar's test (ns), (b) during interactions with equal-sized groups ($n = 17$ colonies of each ant species), Wilcoxon-signed ranks test (***$P < 0.001$), (c) during asymmetrical group interactions with *L. humile* ($n = 20$ workers per nest) and *P. megacephala* ($n = 10$ workers per nest), for 12 trials, paired-samples *t*-test (*$P \leq 0.05$); (d) *L. humile* ($n = 10$ workers per nest) and *P. megacephala* ($n = 20$ workers nest), for 12 trials, Wilcoxon-signed ranks test (***$P < 0.001$). Box-plots (b) and (d) show median, 25 and 75 percentiles, min and max.

Fig. 3. Resource exploitation by each ant species in the absence of a competitor (Baseline): (a) box-plot (median, 25 and 75 percentiles, min and max) showing discovery time of a clumped resource for *Pheidole megacephala* and *Linepithema humile*, Mann–Whitney *U*-test (NS); (b) recruitment to a resource (mean ± SE over 90 min), and (c) retrieval of the resource after 90 min (median, 25 and 75 percentiles, min and max), Mann–Whitney *U*-test (NS).
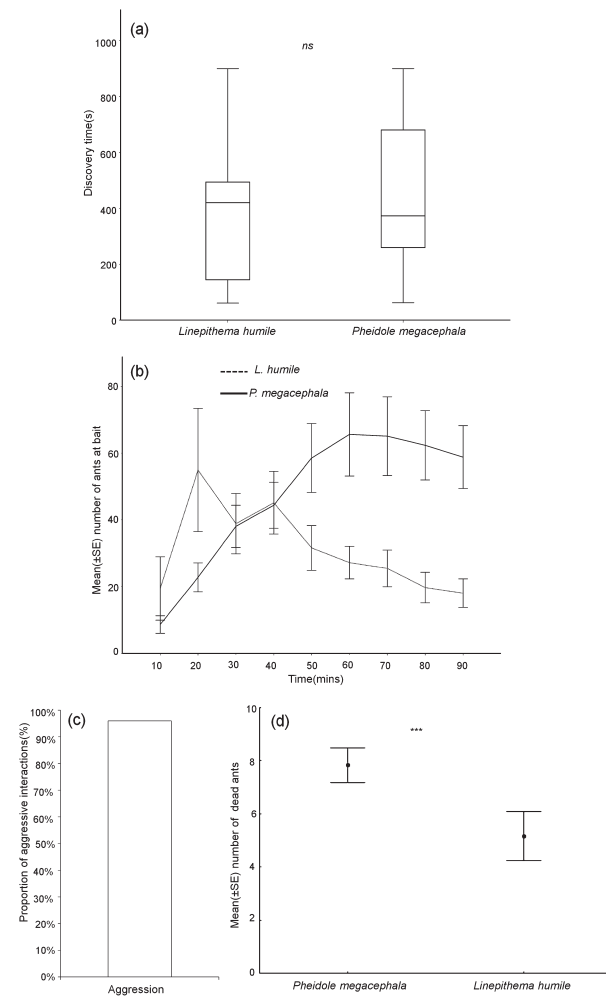

Fig. 4. Foraging parameters and interference during resource competition between *Linepithema humile* and *Pheidole megacephala*: (a) box-plot (median, 25 and 75 percentiles, min and max) showing discovery time of a clumped shared resource for both species, Wilcoxon-signed ranks test (NS); (b) recruitment to a resource (mean ± SE over 90 min) for both *L. humile* and *P. megacephala*; (c) aggression and (d) mortality of both species around the resource, Paired-samples *t*-test (***$P < 0.001$), $n = 17$ colonies of each ant species.

& Silverman, 2005). *Linepithema humile* often loses in individual worker aggressive encounters, but are more successful in group encounters using both physical and chemical defence (Holway, 1999; Buczkowski & Bennett, 2008). This ability to fight better in large groups gives them a competitive edge over most native ant species (Holway, 1999), who do not always fight in groups (e.g. *Tapinoma sessile*-Buczkowski & Bennett, 2008). Our findings showed that both ant species were aggressive across a range of contexts: from one-on-one assays to the resource competition assay. The minors of *P. megacephala* (±2–2.6 mm) are only slightly smaller than Argentine ant workers (±2.5–3.0 mm) (Wild, 2004; Lach *et al.,* 2009; Wetterer, 2012), so the effect of body size on the outcome of the aggressive interactions in the one-on-one and group assays was minimal (Nowbahari *et al.,* 1999) as majors were not included in

these assays. Kirschenbaum and Grace (2008) found that during aggression bioassays when only the minor workers of *P. megacephala* were used, staged interactions with *L. humile* resulted in increased average mortality of *P. megacephala* but survival increased when the soldier caste was present. In this study, *P. megacephala* minor workers were able to defend themselves against equal-sized groups of *L. humile* with approximately four *P. megacephala* killed per trial whereas *L. humile* suffered on average a loss of 8.5 workers per trial. In the resource competition assay, even when *P. megacephala* majors were observed cutting up *L. humile* workers during interactions around the bait (N. Mothapo, pers. obs., also stated in Wetterer, 2012), *L. humile* still only suffered twice the mortality rate compared with *P. megacephala*, even though both suffered much higher mortality rates.

Both *L. humile* and *P. megacephala* were able to rapidly discover, recruit to a resource with high intensity, and retrieve a large amount of food with equal prowess in the absence of a competitor. Although this is a globally known fact for Argentine ants (Human & Gordon, 1996, 1999; Davidson, 1998; Holway *et al.,* 2002a), evidence to support these characteristics for *P. megacephala* has not empirically been tested previously but rather inferred from the predictable foraging behaviour exhibited by most invasive ant species and the fact that *P. megacephala* are populous where they are introduced (Majer & de Kock, 1992; Hoffmann *et al.,* 1999; Vanderwoude *et al.,* 2000; Dejean *et al.,* 2005).

Foraging success is further determined by the ability of ants to defend a shared resource or prevent other ants from having access to it (Andersen & Patel, 1994; Davidson, 1998). Inter-specific competition is one of the key determinants of foraging success in ant communities, allowing for co-existence of different species adapted to different foraging strategies (Fellers, 1987; Davidson, 1997; Santini *et al.,* 2007; Parr & Gibb, 2012). The recruitment of *L. humile* workers during the resource competition assay declined, suggesting that they were deterred from foraging. Depressing the foraging success of native ant species is a mechanism by which *L. humile* typically displaces native ant species in the field (Human & Gordon, 1996, 1999). Here we show that *P. megacephala* uses a similar strategy against *L. humile*, when colony size was controlled for, displacing *L. humile* through lethal aggression. Blight *et al.* (2010) showed that the native dominant ant *Tapinoma nigerrimum* Nylander on the French island of Corsica was a much stronger competitor compared with *L. humile* and was highly efficient at both resource exploitation and interference competition. In their study, *L. humile* even exhibited submissive behaviours when interacting with *T. nigerrimum* at the bait. Therefore, co-occurring ant species with resource preferences that overlap and similar foraging strategies are likely to be highly competitive (Lach, 2005; Kirschenbaum & Grace, 2007, 2008; Lach, 2008), and as most ecologically dominant ant species share similar characteristics they may exclude each other from areas where they co-occur through high competition and aggression. The findings of this study suggest that *P. megacephala* potentially limit the establishment of incipient colonies of Argentine ant into climatically suitable areas of South Africa where it dominates through high interference competition for shared resources.

Numeric dominance has been cited in numerous studies on invasive ants as the most important factor promoting the success of these ants (Holway *et al.,* 2002a; O'Dowd *et al.,* 2003; Abbott *et al.,* 2007; Rowles & O'Dowd, 2007; Vonshak *et al.,* 2012). However, invasive ant species first arrive within a recipient environment as small propagules and do not have this numeric dominance (Hee *et al.,* 2000; Holway *et al.,* 2002a; Tillberg *et al.,* 2007). During the most critical stages of invasion, arrival, and establishment, these small propagules must contend with all abiotic and biotic factors in the recipient environment (Richardson *et al.,* 2000; Holway *et al.,* 2002b; O'Dowd *et al.,* 2003; Walters & Mackay, 2005; Rowles & O'Dowd, 2007). It is known, however, that the presence of ecologically similar species may prevent the establishment of invasive species as well as limit their distribution in areas where they have already invaded (Rowles & O'Dowd, 2007; Blight *et al.,* 2010). Our findings, and those of Blight *et al.* (2010), show that ecologically dominant ant species, such as *T. nigerrimum* (a Dolichoderinae like *L. humile*) and *P. megacephala* (a Generalised Myrmecinae, sensu Andersen, 1995 for functional group classification) can outcompete other ants through highly effective foraging strategies and the ability to defend themselves through high aggression (see also Walters & Mackay, 2005). In Australian communities, areas dominated by the native meat ant species of the genus *Iridomyrmex*, are free from *L. humile* (Walters & Mackay, 2005; Walters, 2006), but these areas also tended to be drier (Thomas & Holway, 2005), and therefore not suitable for *L. humile* (Holway *et al.*, 2002b). Environmental variables such as temperature and humidity have been shown to limit the range expansion of *L. humile* (Holway *et al.,* 2002b; Thomas & Holway, 2005); however, biotic factors such as competition from resident ant species, namely *P. megacephala*, cannot be ignored. Thus, the combination of environmental suitability and the presence of ecologically similar, dominant, and highly competitive native ant species are crucial to limiting the establishment of invasive ant species (Menke & Holway, 2006; Walters, 2006; Menke *et al.,* 2007).

Taking into account the similar ecological niche requirements of *P. megacephala* and *L. humile* (Holway *et al.,* 2002a; Lach *et al.,* 2009), as well as the levels of aggression displayed during interference competition in this study, our findings support the hypothesis that *P. megacephala* potentially limits the spread of *L. humile* along the eastern and northern escarpment of South Africa. Our study also provides further confirmation that competition from ecologically similar and dominant ant species has the potential to significantly affect the invasion success of introduced ant species by limiting their survival and establishment (Richardson *et al.,* 2000). Moreover, our study highlights the importance of studying the biological traits of ants within a recipient environment and those of introduced ant species in order to understand the factors facilitating successful invasion of natural communities.

Acknowledgements

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