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Research article

Large scale unicoloniality: the population and colony structure of the invasive Argentine ant (*Linepithema humile*) in New Zealand

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Abstract. The Argentine ant is native to South America and has spread widely across the globe. In this study, we use genetic analyses and behavioural assays to examine the colony structure of Argentine ants in New Zealand. Diet modification studies were also carried out in order to help identify what factors influence these behavioural assays. There was no aggression observed between any pairings tested across the North Island of New Zealand, though we found that diet manipulations in the laboratory could lead to low levels of aggression between previously amiable Argentine ant nests. The New Zealand population of Argentine ants was characterized by low levels of genetic variation in six microsatellite loci from their nuclear genome. Additionally, the population also lacked significant genetic structuring with no patterns of regional differentiation or isolation by distance. An analysis of molecular variation (AMOVA) found that the majority of genetic variation was present at a nest level (93% of total genetic variance), with little genetic differentiation observed within or between regions (3–4% of total genetic variance). No correlation between aggression and genetic relatedness was observed. This evidence suggests that Argentine ants in New Zealand effectively form a unicolonial population, which is likely the result of colonization from a single source population. As far as we know, this is the first country to have an entirely unicolonial population of Argentine ants.

Keywords: Biological invasions, Formicidae, population genetics, bottleneck, molecular markers.

Introduction

The Argentine ant is an invasive species that has successfully spread from its native range in South America across much of the Mediterranean zones of the globe (McGlynn, 1999; Suarez et al., 2001). It has displaced local ant species (Carpintero et al., 2005; Holway, 2005), as well as many invertebrates (Holway, 1998) and vertebrates (Suarez and Case, 2002). As a social insect, it forms co-operative groups that are able to coordinate food gathering, defence and reproduction. In the native range it has been suggested that these co-operative groups can generally be delineated as discrete nests that are aggressively defensive towards other nearby nests, a population structure termed multicoloniality. However, in the introduced range these groups can be unicolonial (Tsutsui et al., 2000), a term referring to the fact that a colony can occupy multiple nest sites (Hölldobler and Wilson, 1977) and mix freely amongst these nests at large scales (Giraud et al., 2002). This dramatic shift in social structure from the native range to the introduced range is thought by some to at least partly explain how Argentine ants become so ecologically dominant in the introduced range (Holway et al., 1998), though, recent work now argues that the native range also has unicolonial nests that are simply smaller versions of that in the introduced range (Heller, 2004; Pedersen et al., 2006).

The basis of social structure rests on how worker ants interact with related and unrelated conspecifics. This kinship recognition relies on individual ants to compare any newcomers with a recognition template they have formed of acceptable cues (Waldman et al., 1988). This template in ants is thought to rest on cuticular hydrocarbons, or unique chemical cues expressed on the body surface and identifiable via attenuation (Ichinose et al., 2005; Wagner et al., 2000). These cues act as a label which

ants can use to identify nestmates from non-nest mates. There are a number of factors influencing both label and template, including social context, environmental and genetic factors (Bonavita-Cougourdan et al., 1997; Buczkowski and Silverman, 2006). The ability to discriminate nest mates from others is generally thought to be a result of kin selection whereby one actor can increase their own fitness by aiding genetically related individuals (Griffin and West, 2002). However, in the case of some unicolonial populations, individuals who are recognized as nestmates are not genetically related and as such would not be expected to co-operate. Recent work by Tsutsui and others (Tsutsui et al., 2000; Tsutsui et al., 2003) has suggested that introduced populations have undergone a bottleneck and a significant reduction in genetic diversity has erased many recognition alleles. Without these alleles present introduced populations lack enough differentiation between individuals for ants to distinguish kin from non-kin. In contrast, Giraud et al. (2002) argued that a genetic bottleneck is not the only explanation and that many recognition alleles have been purged after the introduction by selection against those with rare alleles. New Zealand is an interesting case because there is genetic evidence to suggest the presence of at least two bottlenecks as they arrived into New Zealand via Australia (Corin et al., 2007).

The situation is more complex than simply being genetically determined as recognition cues are also partly environmentally derived. For example, previous laboratory studies have shown the importance diet can have on the cuticular hydrocarbon profile expressed by an ant (Liang et al., 2001; Silverman and Liang, 2001). However, experimental manipulations have been restricted to manipulating diet using a limited number of prey items, the most common being *Supella* sp. cockroaches (for example Liang et al., 2001). These diet manipulations have been shown to influence the all important cuticular hydrocarbons displayed by ants, and can result in aggression, ultimately leading to colony disassociation (Silverman and Liang, 2001).

Argentine ants were first noted in New Zealand in 1990, in Auckland (Green, 1990). Their distribution is non-continuous, having spread as far south as Christchurch (Fig. 1) and to many of the major cities apparently by human mediated dispersal (Ward et al., 2005). We investigated both genetic and environmental factors in the New Zealand population of Argentine ants in order to answer the following questions; (a) is the New Zealand population characteristically unicolonial; (b); does the pattern of genetic structuring in the New Zealand population correspond to that of an introduced unicolonial population, namely low genetic diversity and little genetic differentiation among nests; and (c) can kinship recognition be influenced by diet in the New Zealand population?

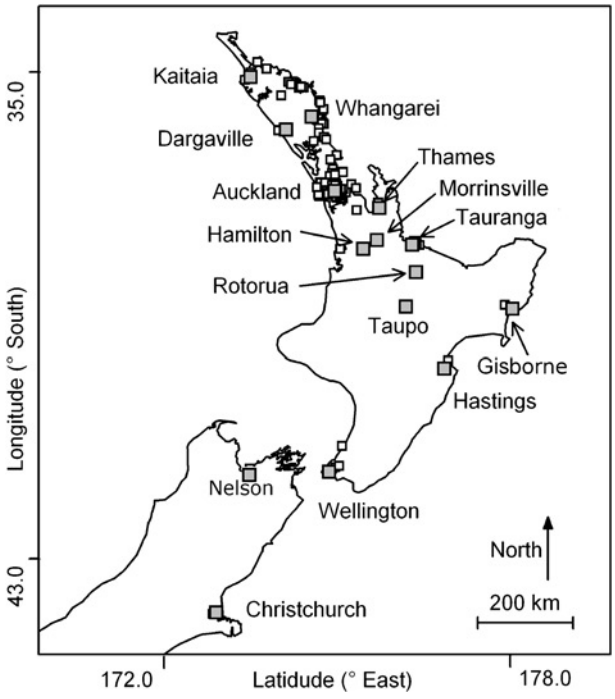

Figure 1. The fifteen locations throughout New Zealand from which Argentine ants were collected in January 2005 (grey squares).The smaller open squares represent the distribution records for Argentine ants in New Zealand.

Methods

Field sites

We collected Argentine ants from 15 urban centers around New Zealand in 2005. Sites were spaced over a 900 km area and encompassed the longitudinal and latitudinal extremes of the known extent of Argentine ants in New Zealand at that time (Fig. 1.). We split our North Island sites into two hierarchical levels, within a city and within a region (Table 1). At each location from between 1 and 9 nests were sampled for ants with nests spatially separated by a minimum distance of 500 m or, if the infestation was too small to allow this, we either took only one sample or two nests as far apart as conditions would allow. We intensively sampled Auckland as it is likely the initial incursion point of Argentine ants in New Zealand (Green, 1990). At each nest we gathered a number of workers as well as any available brood, males, and queens (range 0–8 queens). These individuals were stored in plastic nest boxes (120 mm by 120 mm by 90 mm (high)) with their internal walls painted with Fluon™ (Australian Entomological Supplies) to prevent ant escape. Nests from the North Island were all collected within 3 days of each other and nest material was retained in our attempt to keep conditions as unmodified as possible prior to behavioral assays. The ants were provided with water, but no food material until the initial behavioural assays were completed. The water was provided via absorbent sponge material to keep the nest box moist. 15 ml covered plastic nest tubes (15 mm wide by 95 mm long), 1/3 filled with water and plugged with cotton were also provided to provide a high humidity and dark nest area. Behavioral assays (see below) were performed at maximum 3 days after collection.

Table 1. Sites sampled throughout New Zealand. Nest refers to the number of nests behaviorally assayed. Microsatellites refers to the number of individuals screened for microsatellites from each urban centre. Both the Nelson and Christchurch locations lack any information for region as they were not part of our behavioral assays.

City	Region	Nest(s)	Microsatellites
Kaitaia	North-1	2	5
Whangarei	North-1	2	5
Dargaville	North-1	2	5
Auckland	North-2	9	52
Thames	North-2	1	5
Morrinsville	Central-1	2	5
Hamilton	Central-1	2	5
Rotorua	Central-1	2	6
Tauranga	Central-1	2	6
Taupo	Central-2	2	6
Gisborne	East	2	5
Hastings	South-2	2	5
Wellington	South-1	2	5
Nelson	–	1	4
Christchurch	–	1	11

Behavioral assays

We used the aggression assays developed by Holway et al. (1998) and Suarez et al. (1999) whereby 2 randomly picked workers are paired in a neutral arena for 10 minutes, with interactions between workers scored after 5 seconds and every minute after that for 10 minutes. The interactions were scored on a scale from 0–4 with: Non-aggressive interactions being 0 – ignore and 1 the ants touch and attenuate only with no aggressive response. Aggressive interactions were scored as; 2 the ants touch but quickly retract and avoid each other; 3 discrete attacks between ants such as biting and pulling of antennae and 4 fully fledged and prolonged fighting between ants. Ants were given one hour to acclimatize before we began the trials. For each trial, a naive worker was individually transferred from each nest box to the fluon coated arena (25 mm diameter by 50 mm high) via a fine paintbrush.

Nests were assayed in a nested experiment design. Explicitly this consisted of assaying nests within a city, within a region and between regions. If aggression was absent at each level we randomly picked one nest per hierarchical level from which to continue our analysis. Ten replicates of each combination were conducted, except the fine scale sampling in Auckland where only 5 replicates were possible. Aggression assays conducted following the diet manipulation experiments were of similar nature, the procedures were exactly the same and all pairwise combinations were tested with 5 replicates. If aggression was absent in our trials we conducted additional assays against the New Zealand native ant *Monomorium antarticum* (Smith) and an the introduced *Pheidole rugosula* Forel workers in an attempt to observe maximum levels of *L. humile* aggression.

Genetic analysis

Approximately 30 individual workers were collected from each nest and immediately stored in at least 70% ethanol, at 4°C for genetic analysis. DNA was extracted from a minimum of five worker ants from each of the 15 locations using a slightly modified 5% w/v chelex resin solution extraction protocol (Sepp et al., 1994). Polymerase chain reaction (PCR) was used to amplify six microsatellite loci from the nuclear genome, namely Lhum-11, Lhum-13, Lhum-28, Lhum-35, Lhum-39 (Krieger and Keller, 1999) and Lihu-H (Ingram and Palumbi, 2002). Each 25 µl reaction consisted of 1 µl of template DNA, 0.4 mg/mL of bovine serum albimum (BSA) 1.5 mM $MgCl_2$ 200 µM of each of the four dNTP's, 0.4 µM of a fluorescently labeled forward primer, 0.4 µM of a reverse primer and 0.1 Unit of BioTherm DNA Polymerase (GeneCraft). PCR's were conduced on an Eppendorf 2700 (Applied Biosystems) with a thermal regime consisting of an initial 2 minute denaturing step at 92°C, followed by 35 cycles of 50 seconds at 92°C, 50 seconds at 57°C, 60 seconds at 72°C and a final extension step of 300 seconds at 72°C. PCR products were analyzed on an ABI 3730 Genetic Analyzer and visualized with the aid of Genemapper v3.7 (Applied Biosystems).

Diet manipulation

After initial behavioral assays were performed we transferred the nests to incubators kept at 24°C and with a light/dark cycle of 16/8 hours. Nests were maintained on a standard laboratory diet, consisting of sugarwater every two days and alternating between scrambled eggs or *Lucilia cuprina* Wiedemann flies as a protein source every three days. These conditions were continued for 130 days to remove any trace of previous diet and nest substrate. Behavioral assays were conducted, with no changes in aggression evident. We then randomly picked 12 nests which were assigned to one of three diets. These diets were (a) flies; or six *Lucilia cuprina* flies every two days, (b); crickets, or a black field cricket *Teleogryllus commodus* (Walker) every two days and (c) control, sugarwater, egg and flies as described above. Each nest had all accumulated nest material removed and was standardized to colonies of 200 workers and one queen. For 56 days these nests were maintained before behavioral assays were carried out. One nest suffered the death of a queen and was removed from the analysis.

Statistical analysis

Behavioral assays were statistically analyzed in two ways. Firstly we used mean aggression per behavioral assay for determining the recognition abilities of nests from throughout New Zealand. In our diet studies we relied upon using the maximum aggression from each trial. Though these methods provide similar results (Roulston et al., 2003). We consider that the average aggression scores from a trial are more biologically meaningful with regard to field conditions and interactions. In the field there is often sufficient space for ants to avoid each other and aggressive interactions. We can incorporate this effect to some extent by using the average aggression score in our laboratory assays as it averages the interactions we observed over that period. In contrast we decided to use the maximum aggression score for the laboratory diet manipulation as this experiment essentially had no relevance to field conditions and we felt maximum aggression scores were more likely to answer whether there was a change in ant behaviour. These analyses were performed using the non-parametric Mann-Whitney U test in the software package SPSS v 13.0 (SPSS Inc., Chicago).

Allele discovery curves were generated using the Coleman rarefaction technique (Coleman, 1981) and 10,000 randomization runs as implemented in EstimateS 7.5 (Colwell, 1994–2004). Summary statistics such as expected and observed heterozygosity were obtained using GENEPOP v3.4 (Raymond and Rousset, 1995). We tested for evidence of a bottleneck using allele frequencies using the program Bottleneck v1.202 (Cornuet and Luikart, 1996). This program relies on the observation that bottlenecks cause a 'heterozygosity excess'. In short, as rare alleles are lost rapidly during a bottleneck and they have little effect on heterozygosity, alleles are lost faster than heterozygosity thus creating a 'heterozygosity excess'.

We used an analysis of molecular variation (AMOVA) framework to determine the level of total genetic variation present at each hierarchical level. This was calculated as variation within a nest, between nests in a region and amongst regions using 10,000 permutations in the package Arlequin v3.01 (Excoffier et al., 2005). Addition-

ally, we tested for pairwise differentiation between regions using Fisher's method and statistically tested using a chi-squared test in the package GENEPOP v3.4 (Raymond and Rousset, 1995).We also calculated F_{ST}, the pairwise differentiation of sub-populations in the program GENEPOP v3.4 (Raymond and Rousset, 1995). This data set was combined with the geographical distance and behavioral assay data to look at the relationship between geographical distance and aggression, genetic distance and aggression and isolation by distance. Mantels test was used to test the significance of a linear correlation between these proximity matrices, with 100,000 permutations being used in all cases.

Results

Aggression assays immediately after nest collection

We found no aggression in any of the 602 trials between workers from Argentine ant nests sampled throughout the North Island of New Zealand immediately after collection. Workers spent the whole 10 minutes either attenuating, or ignoring each other. Argentine ants were capable of aggression, as assays descended into mild aggression between *Pheidole rugosula* with leg biting and antennae pulling. There were higher levels of aggression present between the bigger *Monomorium antarticum*, which often attempted, sometimes successfully, to employ its sting against *L. humile*. This aggression was significantly higher than the aggression found between intraspecific trials (Mann-Whitney U, n=622, $p < 0.001$). These behavioural data suggest that all the nests tested belong to a unicolonial population and that the ants sampled are aggressive towards hetero-specifics.

Genetic diversity

We found a total of 19 alleles at six microsatellite loci in the 130 individuals sampled. Two loci were found to be monomorphic (Lhum-35 & Lhum-39) and were excluded from our analysis. The flattening of the allele discovery curve to a plateau suggested that our sample was representative of the genetic variation in the New Zealand population (Fig. 2). Compared to the native range there were fewer alleles (across the 5 comparative loci; 18 in New Zealand and 45 in the native range) (Giraud et al., 2002; Krieger and Keller, 1999; Tsutsui et al., 2000). Moreover, New Zealand has less genetic diversity compared to other introduced populations with 19 alleles over 6 microsatellites compared to an average of 46 in other introduced areas (Buczkowski et al., 2004; Giraud et al., 2002; Ingram and Gordon, 2003; Ingram and Palumbi, 2002; Jaquiery et al., 2005; Krieger and Keller, 1999; Suhr, 2004; Tsutsui et al., 2000). This lack of allelic diversity suggests that New Zealand only contains a small subsample of alleles present elsewhere, particularly compared with the native range.

Observed (H_O) and expected (H_E) heterozygosities were similar (Table 2). No evidence was observed for a 'heterozygosity excess' bottleneck under either the Stepwise Mutation Model or the Infinite Alleles Model (p_{SMM}=0.93750 and p_{IAM}=0.43750). New Zealand displayed a reduced level of heterozygosity compared to other populations, both native (over the comparative loci an average of 0.30 vs. 0.67 (Krieger and Keller, 1999)) and introduced (0.25 vs. 0.43 (Ingram and Palumbi, 2002; Krieger and Keller, 1999; Suhr, 2004)).

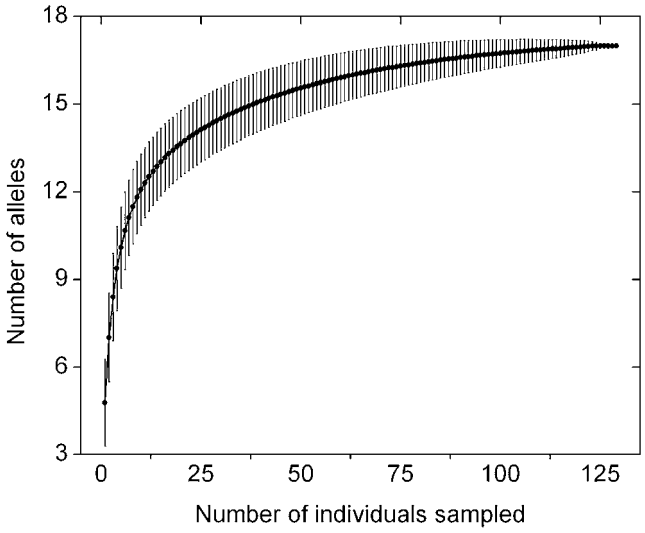

Figure 2. Allele discovery curves for the New Zealand Argentine ant population. Four loci are shown. The x-axis indicates the cumulative number of individuals sampled and the y-axis the cumulative number of different alleles discovered. Grey lines indicate standard error.

Table 2. Summary statistics for the 6 microsatellite loci across the 130 individual ants sampled. The number of alleles found, and both expected (H_E) and observed heterozygosities (H_O) are given.

Loci	Number of Alleles	H_E	H_O
Lhum-11	4	0.08	0.09
Lhum-13	5	0.49	0.43
Lhum-28	4	0.31	0.29
Lhum-35	1	0.00	0.00
Lhum-39	1	0.00	0.00
Lihu-H	4	0.64	0.67
Total	19	0.25	0.25

Population structure and isolation by distance

There appeared to be little differentiation between nests from our New Zealand collections. Most of the total variance of genetic variation was accounted for at the nest level (93.01%) with the variation between nests and nests in a region counting for only 3.89% and 3.10% of total variation respectively. Comparisons of regions also failed to find any F_{ST} differentiation within New Zealand (Table 3). Moreover, we failed to find any lack of significant relationship between the genetic distance

Table 3. Pairwise significance values across all regions and all loci using Fisher's method.

	North-2	Central-1	Central-2	East	South-2	South-1
North-1	0.68073	0.98062	0.31673	0.20123	0.47266	0.26298
North-2		0.33114	0.25280	0.11641	0.15993	0.35079
Central-1			0.71338	0.38405	0.47844	0.95927
Central-2				0.13499	0.75619	0.21697
East					0.76507	0.80426
South-2						0.46392

and geographic distance between nests (Fig. 3a. Mantel test p=0.4541).

The behavioral assays did not show any relationship between the other factors. We found no relationship within the North Island between pairwise behavioral assay scores, and both geographic and genetic distance (Fig. 3.; Mantel test p=0.270 and p=0.990 respectively). All these findings are consistent with a unicolonial population in New Zealand that lacks both behavioral and genetic population structuring.

Aggression assays after diet manipulation

Diet manipulation significantly increased intraspecific aggression of Argentine ants. Aggression was present and significantly higher when compared to within diet assays, between the 'cricket' and 'control' (Mann-Whitney U, n=120, p =0.025) diets as well as the 'cricket' and 'fly' diets (Mann-Whitney U, n=134, $p < 0.001$).These aggressive interactions included many different forms ranging from quick lunging bites to leg pulling and occasionally full combat. No individuals were killed during these interactions, and the proportion of aggressive interactions between these two trial groups was lower than that between interspecific trials (Fig. 4a.), leading to a significant difference in aggression levels (Fig 4b.). Significant differences in aggression were observed between 'cricket diet' and 'control diet' vs. interspecific (Mann-Whitney U, n=105, $p < 0.001$) and between 'cricket diet' and 'fly diet' vs. interspecific (Mann-Whitney U, n=120, $p < 0.001$). No aggression was observed between the different nests within the same diet treatment group. In addition, no aggression was detected between individuals from the 'fly' and 'control' diets. This aggression, however, remained lower than is present between Argentine ants and other species (Fig. 4).

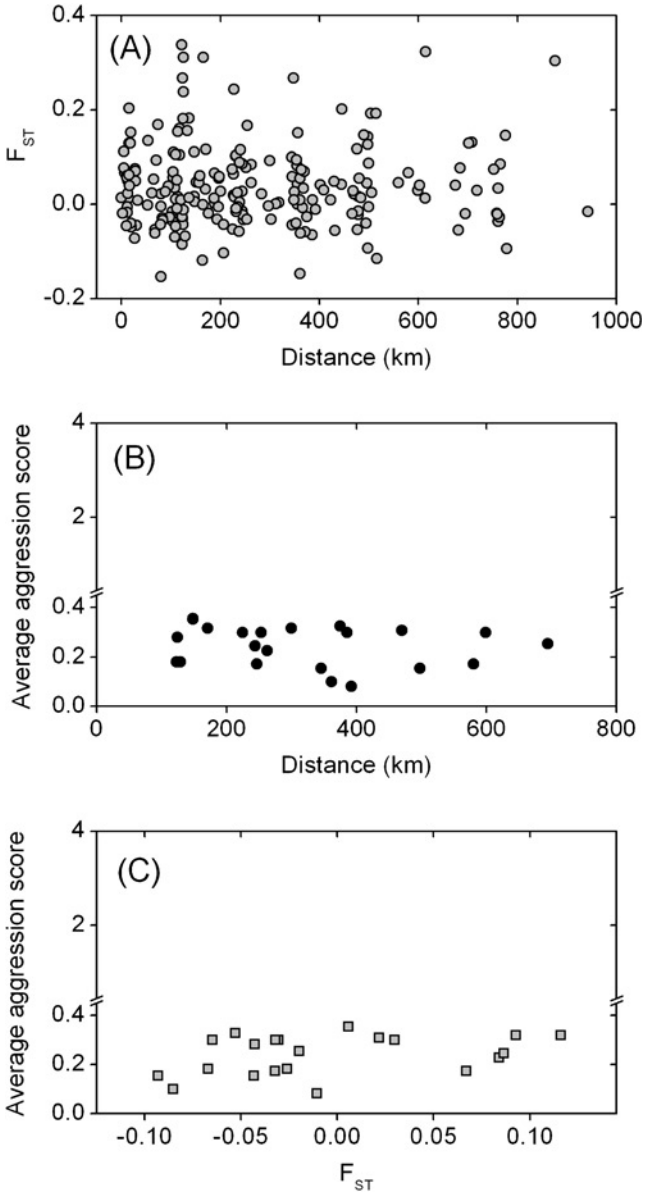

Figure 3. A. The relationship between genetic relatedness and geographical distance. B. The relationship between mean assay score and geographical distance. C. The relationship between mean assay score and genetic relatedness.

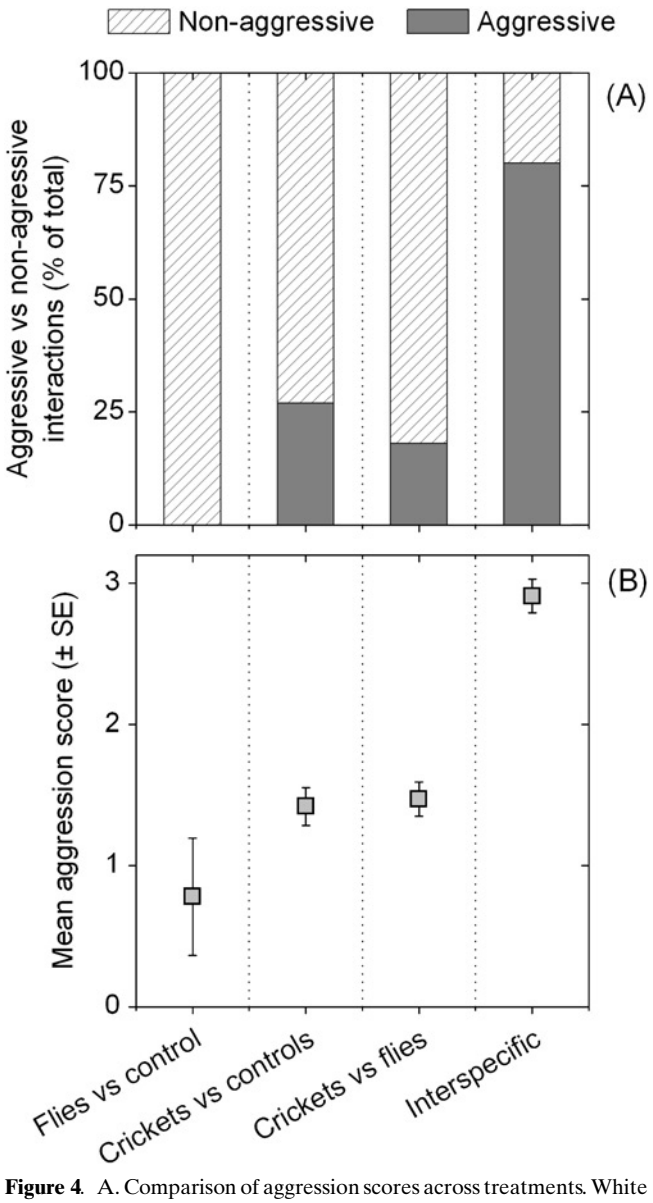

Figure 4. A. Comparison of aggression scores across treatments. White bars denote non-aggressive interactions, black bars represent aggressive interactions. B. Mean aggression score (±1 S.E.) amongst treatments (flies vs. controls n=60, crickets vs. controls n=45, crickets vs. flies n=60).

Discussion

Aggression assays immediately after nest collection

There was a distinct lack of aggression between any of the paired nests across the entire length and breadth of the Argentine ant distribution in the North Island. This is a similar pattern found to that observed throughout much of the introduced range in both Europe and North America (Giraud et al., 2002; Tsutsui et al., 2000). The absence of aggression suggests there is a unicolonial population of Argentine ants spread across almost 700 km in the North Island of New Zealand. As far as we know this is the first country to have an entirely unicolonial population. Unicoloniality has been associated with increased success of Argentine ants in competitive interactions with other species (Holway, 1999; Holway et al., 1998). It appears that this reason for success may be the case in New Zealand as well, with the presence of Argentine ants often associated with a lack of other ants species (Brightwell, 2002, Corin, S. pers. obs.).

Genetic diversity

In general, allelic diversity in New Zealand was low compared to other populations around the world. This coupled with the lower levels of heterozygosity is evidence that a bottleneck has at some stage caused such a reduction. There was some evidence to suggest that this bottleneck has not been extremely 'tight'. In the case of a severe bottleneck allelic diversity would be expected to decrease faster than heterozygosity and hence lead to an 'heterozygosity excess' (Luikart and Cornuet, 1998; Maruyama and Fuerst, 1985), which appears not to be the case in New Zealand. Specifically, the presence of multiple alleles is strong evidence that there was not a particularly tight bottleneck. In Argentine ants almost all queens are inseminated from the sperm of a single male (Keller and Passera, 1992; Krieger and Keller, 2000). Therefore, in the founding population of Argentine ants there either needed to be more than a single inseminated founding queen, or the presence of multiple males. Given the fact that there are at least five alleles at a single locus it seems unlikely that this diversity would be able to arise in New Zealand through mutation, particularly given that Argentine ants are a relatively recent arrival in the country (Green, 1990).

If there has not been a severe bottleneck then how can we explain the low levels of genetic diversity? There are a number of potential reasons for this. First, there may have been a severe bottleneck we have not detected. We can only detect population bottlenecks from allele frequency data recently after they occur (Cornuet and Luikart, 1996). Though Argentine ants were detected in New Zealand in 1990 (Green, 1990), they may have been present for much longer and in which time recovered from the bottleneck. Additionally, our test relied on the assumption that the population remains relatively small. A large dramatic increase in population size may mask any 'excess heterozygosity'. Secondly, there is the chance that there simply has not been a severe bottleneck. There is now evidence from mtDNA that suggests New Zealand's population of Argentine ants probably originated in Australia (Corin et al., 2007), which has since spread throughout New Zealand. Therefore the New Zealand population may have undergone two bottlenecks, one upon introduction in Australia and one into New Zealand. Australia has had a long history of Argentine ants with their first detection occurring in 1939 (Jenkins, 1961). Therefore, the bottleneck into Australia may have

been severe and reduced both allelic diversity and levels of heterozygosity. Over time, an increase in the Australian population may have masked the 'excess heterozygosity' created by a bottleneck, but left relatively few alleles in the population. If the bottleneck into New Zealand was 'wide' there would be little change in heterozygosities, with only the potential loss of a few rare alleles. All explanations need not be mutually exclusive and together potentially explain how New Zealand has such a low number of alleles present indicating a significant bottleneck yet no evidence for this bottleneck in allele frequencies. Perhaps by examining a larger number of loci in New Zealand and Australia may shed light on some of these questions.

Population structure and isolation by distance

Argentine ants disperse by one of two methods. Firstly they will inherently disperse by a slow fission as groups of workers and queens bud off from the main nest to form a nest of their own (Newell and Barber, 1913; Suarez et al., 2001). Secondly, fragments of populations can be transferred via human mediated dispersal (Suarez et al., 2001). The lack of genetic differentiation amongst nests in New Zealand and the absence of isolation by distance is congruent with Argentine ants rapidly spreading mainly via human mediated dispersal, as suggested by Ward et al. (2005). The lack of any significant relationship between distance and aggression levels also corroborates this finding.

Finally, there appears to be no relationship between genetic relatedness and aggression in New Zealand. This was a surprising result given the strong findings of others linking genetic differentiation with aggression in both the native and introduced range (Tsutsui et al., 2003). We believe a likely explanation for our results is that they are an artifact of low genetic diversity and low aggression between behavioral pairings in New Zealand. Though we lack any behavioral data from the two South Island localities, we can infer from the lack of genetic structuring that these two Argentine ant populations are also likely to be part of the unicolonial population of the North Island.

Aggression assays after diet manipulation

The populations of Argentine ants we examined within New Zealand were non-aggressive towards one another and simulated what one would expect if they accepted each other as nestmates, despite the large geographical distances separating them. Diet has been shown previously by other authors to influence this recognition system (Liang et al., 2001; Silverman and Liang, 2001). We also found that diet did have an impact on recognition with previously amicable interactions becoming aggressive in some cases. We found this result surprising given our personal observations that the field diet of some nests appeared to differ widely. Some nests seemed to consist primarily on scale honeydew, whereas at other nests, no honeydew producers were observed and we could only guess at the nests staple food. We believe the best explanation for our results is that the diets containing no joint food items can become aggressive to one another, but any diet with a small amount of overlap in common food items generally remain non-aggressive. However, other potential explanations are possible, for example, workers that are given a single food source may show unnaturally high concentration of very specific hydrocarbons.

Though our diet manipulations did not produce as dramatic a change as some previous studies (Silverman and Liang, 2001), our results do suggest that the influence of diet may be more widespread than previously thought. The change in aggression due to diet has been suggested by some to only be present in genetically depauparate populations. For example two populations were studied by Buczkowski and Silverman (2006), one with a higher level of genetic diversity than the other. They found that changes in aggression levels were limited mainly to the low genetic diversity population. As New Zealand has comparative levels of genetic diversity to that population (23 alleles at 7 loci; Buczkowski et al., 2004), we cannot rule out the possibility that we would not have found the same pattern had more genetic diversity been present in New Zealand.

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References

Bonavita-Cougourdan A., Bagnères A.G., Provost E., Dusticier G. and Clément J.L. 1997. Plasticity of the cuticular hydrocarbon profile of the slave-making ant *Polyergus rufescens* depending on the social environment. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **116**: 287 – 302

Brightwell J.R. 2002. Exploitative and interference competition in Argentine ants (*Linepithema humile*). Wellington: Victoria University of Wellington.

Buczkowski G. and Silverman J. 2006. Geographic variation in Argentine ant aggression behaviour mediated by environmentally derived nestmate recognition cues. *Anim. Behav.* **71**: 327 – 335

Buczkowski G., Vargo E.L. and Silverman J. 2004. The diminutive supercolony: the Argentine ants of the southeastern United States. *Mol. Ecol.* **13**: 2235 – 2242

Carpintero S., Reyes-López J. and Arias De Reyna L. 2005. Impact of Argentine ants (*Linepithema humile*) on an arboreal ant community in Doñana National Park, Spain. *Biodiversity Conserv.* **14**: 151 – 163

Coleman B.D. 1981. On random placement and species-area relations. *Mathemat Biosciences* **54**: 191 – 215

Colwell R.K. 1994 – 2004. EstimateS: statistical estimation of species richness and shared species from samples. Persistent URL: http://purl.oclc.org/estimates.

Corin S.E., Lester P.J., Abbott K.L. and Ritchie P.A. 2007. Inferring historical introduction pathways with mitochondrial DNA: the case of introduced Argentine ants (*Linepithema humile*) into New Zealand. *Divers. Distrib.: in press*

Cornuet J.M. and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001 – 2014

Excoffier L., Laval G. and Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47 – 50

Giraud T., Pedersen J.S. and Keller L. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. *PNAS* **99**: 6075 – 9

Green O.R. 1990. Entomologist sets new record at Mt Smart *Iridomyrmex humilis* established in New Zealand. *The Weta* **13**: 14 – 15

Griffin A.S. and West S.A. 2002. Kin selection: fact and fiction. *Trends Ecol. Evol.* **17**: 15 – 21

Heller N.E. 2004. Colony Structure in introduced and native populations of the invasive Argentine ant, *Linepithema humile*. *Insect Soc.* **51**: 378 – 386

Hölldobler B. and Wilson E.O. 1977. The number of queens: an important trait in Ant evolution? *Naturwissenschaften* **64**: 8 – 15

Holway D.A. 1998. Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oceologia* **116**: 252 – 258

Holway D.A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology* **80**: 238 – 251

Holway D.A. 2005. Edge effects of an invasive species across a natural ecological boundary. *Biol. Conserv.* **121**: 561 – 567

Holway D.A., Suarez A.V. and Case T.J. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* **282**: 949 – 952

Ichinose K., Cerda X., Jean-Philippe C. and Lenoir A. 2005. Detecting nestmate recognition patterns in the fission-performing ant *Aphaenogaster senilis*: A comparison of different indices. *J. Insect Behav.* **18**: 633 – 650

Ingram K.K. and Gordon D.M. 2003. Genetic analysis of dispersal dynamics in an invading population of Argentine ants. *Ecology* **84**: 2832 – 2842

Ingram K.K. and Palumbi S.R. 2002. Characterization of microsatellite loci for the Argentine ant, *Linepithema humile*, and their potential for analysis of colony structure in invading Hawaiian populations. *Mol. Ecol. Notes* **2**: 94 – 95

Jaquiery J., Vogel V. and Keller L. 2005. Mulitlevel genetic analyses of two European supercolonies of the Argentine ant, *Linepithema humile*. *Mol. Ecol.* **14**: 589 – 598

Jenkins C.F.H. 1961. The Argentine ant (*Iridomyrmex humilis*) in Western Australia. *J. Dept. Agric. W. Australia* **J 2**: 683 – 703

Keller L. and Passera L. 1992. Mating systems, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. *Behav. Ecol. Sociobiol.* **31**: 359 – 366

Krieger M.J.B. and Keller L. 1999. Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Mol. Ecol.* **8**: 1075 – 1092

Krieger M.J.B. and Keller L. 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. *Mol. Ecol.* **9**: 119 – 126

Liang D., Blomquist G.J. and Silverman J. 2001. Hydrocarbon-released nestmate aggression in the Argentine ant, *Linepithema humile*, following encounters with insect prey. *Comp. Biochem. Physiol.* **129**: 871 – 882

Luikart G. and Cornuet J.M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* **12**: 228 – 237

Maruyama T. and Fuerst P.A. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* **111**: 675 – 689

McGlynn T.P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. *J. Biogeogr.* **26**: 535 – 548

Newell W. and Barber T.C. 1913. The Argentine ant. *USDA Bureau of Entomology Bulletin* **122**

Pedersen J.S., Krieger M.J.B., Vogel V., Giraud T. and Keller L. 2006. Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution* **60**: 782 – 791

Raymond M. and Rousset F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and economics. *J. Hered.* **86**: 248 – 249

Roulston T.H., Buczkowski G. and Silverman J. 2003. Nestmate discrimination in ants: effect of bioassay on aggressive behavior. *Insect Soc.* **50**: 151 – 159

Sepp R., Szabó I., Uda H. and Sakamoto H. 1994. Rapid techniques for DNA extraction from routinely processed archival tissue for use in PCR. *J. Clin. Pathol.* **47**: 318 – 323

Silverman J. and Liang D. 2001. Colony disassociation following diet partioning in a unicolonial ant. *Naturwissenschaften* **88**: 73 – 77

Suarez A.V. and Case T.J. 2002. Bottom-up effects of persistence of a specialist predator: ant invasions and horned lizards. *Ecol. Appl.* **12**: 291 – 298

Suarez A.V., Holway D.A. and Case T.J. 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. *PNAS* **98**: 1095 – 1100

Suarez A.V., Tsutsui N.D., Holway D.A. and Case T.J. 1999. Behavioral and genetic differntiation between native and introduced populations of the Argentine ant. *Biol. Invasions* **1**: 43 – 53

Suhr E.L. 2004. The making of an invader: genetic structure and behaviour of the Argentine ant (*Linepithema humile*) in Australia. Monash: Monash University. 62 p.

Tsutsui N.D., Suarez A.V. and Grosberg R.K. 2003. Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *PNAS* **100**: 1078 – 1083

Tsutsui N.D., Suarez A.V., Holway D.A. and Case T.J. 2000. Reduced genetic variation and the success of an invasive species. *PNAS* **97**: 5948 – 5953

Wagner D., Tissot M., Cuevas W. and Gordon D.M. 2000. Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *J. Chem. Ecol.* **26**: 2245 – 2257

Waldman B., Frumhoff P.C. and Sherman P.W. 1988. Problems of kin recognition. *Trends Ecol. Evol.* **3**: 8 – 13

Ward D.F., Harris R.J. and Stanley M.C. 2005. Human-mediated range expansion of Argentine ants *Linepithema humile* (Hymenoptera: Formicidae) in New Zealand. *Sociobiology* **45**: 401 – 407

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