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ORIGINAL PAPER

Behaviourally and genetically distinct populations of an invasive ant provide insight into invasion history and impacts on a tropical ant community

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Abstract Populations of invasive species often exhibit a high degree of spatial and temporal variability in abundance and hence their effects on resident communities. Here, we examine behavioural, genetic and environmental factors that influence variation in populations of the yellow crazy ant, *Anoplolepis gracilipes*, on the remote Nukunonu Atoll of Tokelau, Pacific Ocean. Behavioural assays revealed high levels of aggression between two groups of yellow crazy ants from different islands, and genetic analysis confirmed the presence of two distinct populations with unique mitochondrial (mt)DNA haplotypes, designated A and D. The two populations likely resulted from two separate invasion events. The populations exhibited significant differences in abundance of *A. gracilipes*, with a mean sevenfold difference in relative abundance between the two main haplotypes. The higher density haplotype D population coexisted with 50% fewer other ant species and altered ant community composition. Vegetation composition was also significantly different on islands harbouring the two populations. The results suggest genetic differences could play a role in the spatial and temporal variation in the effect of the yellow crazy ant on a small oceanic atoll. We could not differentiate between genetic effects and effects of vegetation. However, our results indicate that spatial variability in behaviour and impacts within populations of invasive species could be in part due to genetic differences, and play a substantial role in influencing the outcome of biological invasions.

Keywords Invasive ants · *Anoplolepis gracilipes* · Haplotype · Intraspecific aggression · Tokelau · Differential impacts · Invasion history

Introduction

Population explosions of established invasive species occur due to the right combination of attributes derived from both the recipient environment and the invader itself (Simberloff 1995; Hewitt and Huxel 2002; Davis et al. 2005). However, not all exotic species that expand their introduced range also become pestiferous, and factors involved in the early stages of invasion (successful arrival and establishment) may be different to those at the final stage of an invasion (spread and increase in abundance). Many tramp ant species have the capacity to become invasive, but of approximately 30–40 acknowledged tramp ants (Hölldobler and Wilson 1990; Passera 1994;

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Morrison 1997), only several species have recently undergone sufficient post-establishment population growth to become problematic on oceanic islands. McGlynn (1999) claims that Pacific islands host more transferred ant species than any other biogeographic region. Similar to other invasive taxa, they commonly exhibit low population abundances for long periods of time, then boom, and bust again (Brown 1954; Clark 1982; Hoffmann 1998; Sakai et al. 2001; Simberloff and Gibbons 2004; Abbott 2004). It is the mechanisms responsible for these booms and busts that are poorly understood.

Contrary to the idea that lack of genetic variability is detrimental to population growth and survival of most animals (Quattro and Vrijenhoek 1989), genetic homogeny and subsequent behavioural benevolence has been implicated in the success of some invasive ant species. The formation of expansive supercolonies by Argentine ants, *Linepithema humile* (Mayr), in North America and Europe has been attributed in part to genetic bottlenecks that produce less variability at multiple microsatellite loci, and 'genetic cleansing' at recognition loci (Suarez et al. 1999; Tsutsui et al. 2000; Giraud et al. 2002). A study into the geographical diversity of haplotypes in invasive *Phragmites* populations in the United States similarly indicates that invasiveness is due to the colonisation and spread of distinct genotypes from Europe (Saltonstall 2002). However, on Christmas Island, the formation of high-density supercolonies of the yellow crazy ant *Anoplolepis gracilipes* (Smith) was thought to be linked to their mutualistic association with honeydew-producing scale insects and therefore vegetation, fuelling high tempo foraging by providing a rich source of carbohydrates. In conjunction with genetic factors, biotic associations may well amplify the success and impacts of invasive ants on islands (O'Dowd et al. 2003; Abbott 2004).

The yellow crazy ant, *Anoplolepis gracilipes*, has been ranked as one of the world's worst invaders (Lowe et al. 2000). It has achieved a pantropical distribution, and is present on most Pacific islands in low densities (Wilson and Taylor 1967; O'Dowd et al. 2003; Wetterer and Vargo 2003; Lester and Tavite 2004; Wetterer 2005). On Tokelau, a small Pacific nation made up of three low-lying coral atolls, *A. gracilipes* has recently reached extremely high numbers in villages on two atolls, but is absent from a third atoll (Lester and Tavite 2004). *Anoplolepis gracilipes* was recorded from Tokelau in 1934 (Wilson and Taylor 1967), but Lester and Tavite (2004) suggested that populations on the inhabited islands of Nukunonu and Fakaofo Atolls had arrived recently, and might represent a separate invasion event. Such an invasion history can be inferred by using population genetic data derived from the mitochondrial genome (Waldman et al. 2004). Mitochondrial DNA (mtDNA) evolves rapidly and its haploid nature and maternal mode of inheritance make it an ideal marker for studying the history of the female lineages in populations (Avise 1991; Ballard and Rand 2005). For ants that are frequently moved between geographical locations and that require a queen to found a nest and colony, mtDNA data can help us trace invasion pathways effectively.

On Nukunonu Atoll the invasion of yellow crazy ants appeared to have changed the composition and dynamics of both existing ant and other invertebrate communities (Lester and Tavite 2004). More recent observations revealed what seemed like an asymmetry in the relative abundances and nest densities of *A. gracilipes*, associated with what Lester and Tavite suggested was the 'new' invasion on inhabited islands, and a probable 'old' invasion on uninhabited islands. We quantified the behavioural, genetic and environmental factors that might influence the success of the yellow crazy ant on two atolls of Tokelau. We hypothesized that the arrival of new genetic material into existing populations is a possible mechanism for the spatial and temporal variation in behaviour, abundance and impacts observed in this invasion, and indeed many other invasive species (Sakai et al. 2001; Simberloff and Gibbons 2004).

Materials and methods

Tokelau lies in the humid tropics, ~483 km north of Western Samoa and between latitudes 8°30′ and 9°28′S and 171°12′ and 172°32′W. It experiences a wet tropical rainforest climate

(Mueller-Dombois and Fosberg 1998), where the mean annual temperature is 28°C, July being the coolest month, and between December and March the warmest. The small country consists of three atolls, Fakaofo, Nukunonu and Atafu, each with between 38 and 47 low-lying coral islands that encircle relatively shallow lagoons. The yellow crazy ant, *A. gracilipes*, has been observed on only two atolls: Nukunonu, and Fakaofo, where seven out of about 42 islands, and two out of about 51 islands are infested, respectively. Of the 28 ant species recorded from Tokelau (Abbott et al. 2006), none are considered native to Tokelau. The atolls are serviced by a ship that originates in Samoa and visits the atolls every 14–20 days. Data for this study were collected over two visits to Nukunonu Atoll, the first in November/December 2004 and the second in June/July 2005.

Intraspecific aggression

We examined for distinct populations of *A. gracilipes* by using intraspecific aggression assays. Lack of intraspecific aggression within or between colonies of unicolonial ant species has been a behavioural mechanism central to recent hypotheses explaining the disproportionate abundance of invasive ant species in their introduced range (Holway et al. 1998a, b; Tsutsui et al. 2000; Giraud et al. 2002; Holway et al. 2002). A lack of aggression may indicate, among other things, that: (a) interconnected nests that cover a large area have originated from a single focal colony or from one introduction, (b) ants introduced to the same location, but at different times, are from the same source population, or (c) ants in different locations have been introduced (whether at different or at the same time) from the same source population.

From six *A. gracilipes* infested islands on Nukunonu Atoll, we collected workers, queens, brood and nest material from two intact nests using a petrol-driven garden vacuum. The nests were at least 50 m apart. The islands were Nukunonu, which is inhabited and connected by a concrete bridge to Motuhaga; Te Puka I Mua and Tokelau Islands, both which are visited frequently, and Lalo Island, Te Fala and Pukapuka, which is visited less often and used primarily for collecting coconuts (Fig. 1).

After transfer into tubs with their rims coated with Tanglefoot® to prevent escape, all nest contents were left to acclimate and settle before aggression trials. We conducted intraspecific aggression assays (sensu Suarez et al. 1999), between pairs of individual worker ants from the different nests. We carried out 10 trials for each of 21 combinations of nest origin, including between nests on the same island and between the first nests collected from each island with all other islands. Pairs of ants were placed in fluon-coated 80 ml vials (arena 40 mm diameter), and the highest level of aggression between the two ants during the interaction was recorded after 10 min. Interactions were scored as: 0 = ignore; 1 = touch, antennation and grooming; 2 = avoid; 3 = aggression, dorsal flexion for chemical defense, biting or pulling legs and antennae, repeated back and forth jerking; 4 = fighting, prolonged aggression, sparring, charging, biting and chemical defense. Levels 0–2 are considered non-aggressive behaviour, and 3–5 aggressive behaviour. We used the mean of all completed replicates as the level of intraspecific aggression for that combination. At least 20 workers from each nest were preserved in ~95% ethanol for genetic analysis. All assays were conducted within 40 h after collection from the field.

A Kruskal–Wallis rank sum test was used to determine differences in aggression levels both within island nest groups, and between islands on Nukunonu Atoll.

Genetic analysis

To examine intraspecific genetic variation among nests of *A. gracilipes*, we extracted DNA from 80 individual foragers subsampled from collections for aggression assays and community composition analysis, using a petrol-driven vacuum and pitfall traps, respectively. We initially sequenced two individuals from each collection (nest or pitfall trap), then haphazardly subsampled from each collection as resources allowed. In addition, we collected *A. gracilipes* from Fakaofo Atoll, and Upolu and Manono islands, Samoa to determine whether *A. gracilipes* from Nukunonu Atoll has

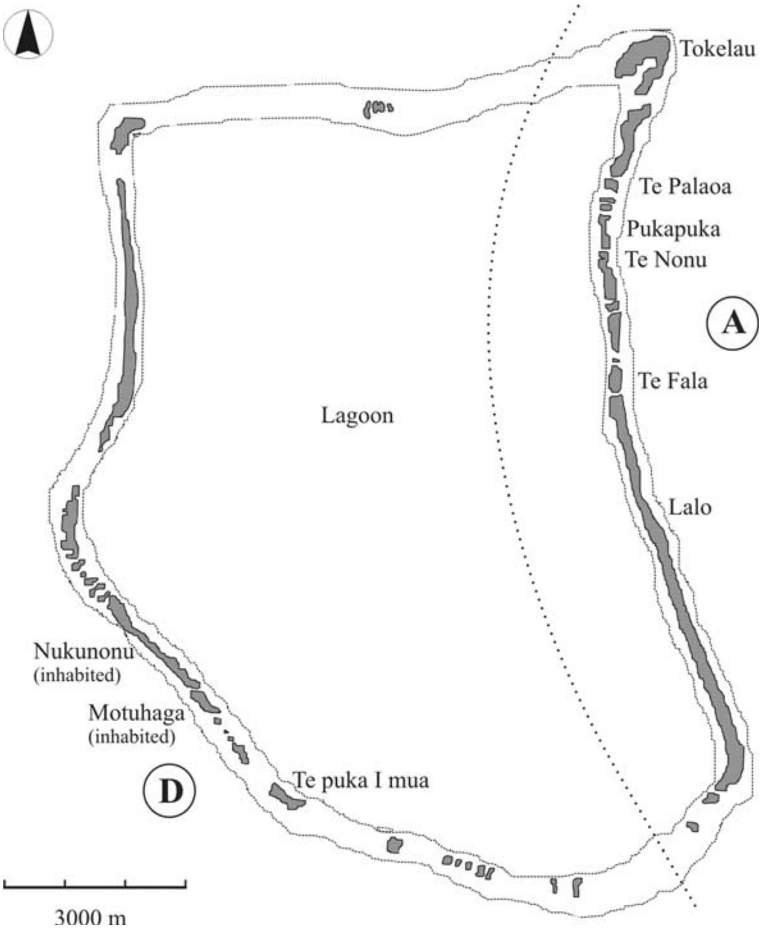

Fig. 1 Map of Nukunonu Atoll, Tokelau, showing division of behaviourally and genetically distinct populations of *Anoplolepis gracilipes*. *A. gracilipes* individuals on the left hand side of the *dotted line* (haplotype D) represent the 'new' invasion, and those on the right hand side of the *dotted line* the 'old' invasion. Labelled islands are those used in intraspecific aggression trials, genetic analysis or analysis of ant community composition. Nukunonu and Motuhaga are connected by a concrete bridge. *Faint double lines* surround islands denote the edge of the reef

haplotype affiliations with populations from its nearest neighbours and direct transport pathways.

Total genomic DNA was extracted by homogenising single ants in lysis buffer containing 10 mM Tris–HCl (pH 8.0), 50 mM NaCl, 10 mM EDTA and 0.2% SDS. Samples were incubated overnight at 50°C with 0.5 mg/ml proteinase K and then purified using a standard phenol and chloroform method. DNA was precipitated using ethanol and then resuspended in TE buffer (10 mM Tris pH8.0, 1.0 mM EDTA) for storage at 4°C. We used PCR to amplify a 460-bp fragment of the mitochondrial locus apocytochrome *b* (*cob*) from each sample using the primers CB1 5′-TATGTACTACCATGAGGACA AATATC-3′ and CB2 5′-ATTACACCTCCTAATTTATT AGGAAT-3′ (Chiotis et al. 2000). Reactions were performed in 25 μl volumes containing 1 μl of DNA template, 67 mM Tris–HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.4 mg/ml BSA, 200μM each dNTP, 0.4μM each primer and 0.1 Units of BioTherm DNA Polymerase (GeneCraft). A typical PCR profile consisted of 35 cycles of 94°C for 10 s, 35°C for 15 s and 72°C for 1 min, using a GeneAmp 2700 thermal cycler (Applied Biosystems). We purified the PCR products using High Pure purification columns (Roche) and then sequenced these fragments directly using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with an ABI 3730 Genetic Analyzer. The sequence data were edited manually and then aligned by ClustalX (Thompson et al. 1997) executed in MEGA 3.0 (Kumar et al. 2004). Each fragment was translated using the invertebrate mitochondrial genetic code, and since none contained premature stop codons, it is assumed that they are all of mitochondrial origin. To visualise the relationships between the haplotypes, we used the software TCS (Clement et al. 2000) to construct a parsimony haplotype network diagram.

Ant community composition

Pitfall traps were used to examine species richness, ant community composition and assess the relative abundance of *A. gracilipes* from six *A. gracilipes*-invaded islands (Nukunonu, Motuhaga, Te Puka I Mua, Te Fala, Pukapuka and Tokelau Island) and two uninvaded islands (Te Nonu and Te Palaoa). In June 2005, two 15 m × 15 m plots were placed in the interior of each island, in which five pitfall traps were placed randomly. The pitfall traps were plastic cups 9 cm tall, 7.5 cm diameter at the top and tapered to 5 cm diameter at the bottom. The cups were placed flush with the ground surface, one-third filled with Gault's solution, an insect killing agent and preservative (Walker and Crosby 1988), and left out for 24 h before collection. We did not leave the pitfalls any longer than 24 h due to the speed at which they often fill up with *A. gracilipes* (Lester and Tavite 2004), and rain, rats and falling coconuts jeopardised their efficacy after 24 h. Ants were sorted from pitfall catches and identified to species using the keys of Wilson and Taylor (1967) and Bolton (1977). Nomenclature, where modified since Wilson and Taylor (1967), follows Wetterer and Vargo (2003).

We used the mean number of each ant species in pitfall traps to analyse the similarity in community composition between; (a) *A. gracilipes*-invaded islands and uninvaded islands, and (b) islands with confirmed different haplotypes. We used non-metric MDS (using 30 restarts to reduce stress) and ANOSIM procedures on $\log_{10}(x + 1)$-transformed data, and similarity matrices were calculated using the Bray–Curtis method to acknowledge common species (Primer ver. 5.2.9; Clarke and Warwick 1994).

Floristic composition

Vegetation type is thought to influence *A. gracilipes* populations primarily through food supply, by providing sugary substances which foragers feed on directly, or by supporting populations of honeydew-producing insects (Haines and Haines 1978; Abbott 2005). However, these factors will also influence populations of other resident ant species and could contribute toward differences in ant communities between islands. We compared floristic composition by quantifying stem density of tree species present on the interior of each island of used for the ant community analysis. We recorded the species of all stems >5 cm dbh within two 50 m × 20 m transects placed randomly in the interior of each ten islands on Nukunonu Atoll, and tested the difference in floristic composition between; (a) *A. gracilipes*-invaded islands and uninvaded islands, and (b) islands with confirmed different *A. gracilipes* haplotypes. We used non-metric MDS (using 30 restarts to reduce stress) and ANOSIM procedures on $\log_{10}(x+1)$-transformed data, and similarity matrices were calculated using the Bray-Curtis method to acknowledge common species (Primer ver. 5.2.9; Clarke and Warwick 1994).

Results

Intraspecific aggression

We observed high levels of intraspecific aggression between workers from different islands on Nukunonu Atoll (Kruskal–Wallis $\chi^2 = 14.624$, $df = 2$, $P = 0.0007$). There were two distinct groups of non-aggressive *A. gracilipes* workers: those from nests on Tokelau, Pukapuka, Te Fala and Lalo islands, and those from Nukunonu, Motuhaga and Te Puka I Mua islands (Fig. 1). Ants collected from these two groupings were generally further than 6 km apart, and were aggressive toward one another 95.75% of the time (aggression score of ≥3; Fig. 2), while ants from neighbouring islands within the groupings were generally friendly to one another.

Genetic analysis

Genetic analysis of ants from Nukunonu and Fakaofo Atolls in Tokelau and Manono and Upolu Islands in Samoa revealed four haplotypes designated A, B, C and D (Fig. 3). On Nukunonu, haplotypes fell out in two main groups, which

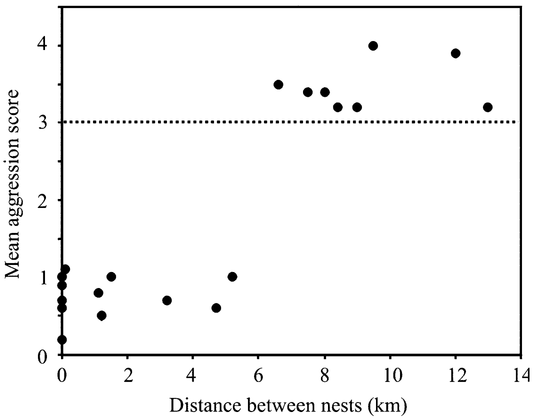

Fig. 2 The mean of the highest aggression scores reached over a 10 min period for combinations of nests on Nukunonu Atoll. Score of 3 or higher indicates aggressive behaviour

were the same groups found during the behavioural aggression analysis (Fig. 1). The four haplotypes were characterised by eight variable nucleotides for a 401-bp fragment of co*b*. Three of the substitutions were A↔G transitions and five were C↔T transitions. Seven of the substitutions were synonymous changes, that is, they do not change the amino acid sequence of the encoded protein, while one was a non-synonymous change at a second codon position, resulting in an amino acid change.

Three of the haplotypes, A, C and D, were found within Nukunonu Atoll. These can be classified into two main groupings, that is, A and C haplotypes versus D haplotype, because haplotype C is closely related to haplotype A, differing at one site only. Haplotype C was found in one individual only, collected in a pitfall trap on Pukapuka Island. On Nukunonu Atoll, haplotype A was found on Tokelau island ($n = 6$ individuals), Pukapuka ($n = 15$), Te Fala ($n = 8$) and Lalo Islands ($n = 10$), while haplotype D was found on Nukunonu ($n = 14$), Motuhaga ($n = 2$) and Te Puka I Mua Islands ($n = 7$). In Samoa, haplotype A ($n = 8$) and the closely related haplotype B ($n = 2$) were present, as well as haplotype D ($n = 1$). Haplotype B differs from haplotype A at one site only, and was found in two different individuals sampled from the same nest. On Fakaofo Atoll in Tokelau, both haplotype A ($n = 2$) and haplotype D ($n = 5$) were found.

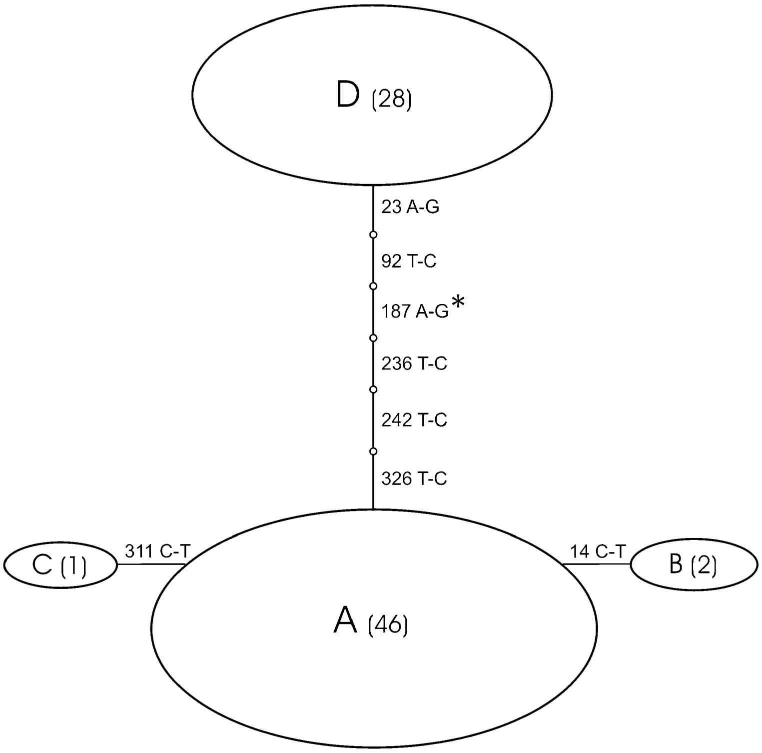

Fig. 3 Parsimony network summarising the relationships between our sequenced haplotypes. Nucleotide substitutions, and their position in the sequenced fragment, are shown in the diagram as the position number followed by the nucleotide change. All substitutions were synonymous, except one, which is indicated in the diagram with an *asterisk*. Haplotype B was found on Upolu island (Samoa) only, while haplotype C was found on Pukapuka island on Nukunonu Atoll only. Haplotypes A and D were present on islands on Nukunonu Atoll, islands on Fakaofo Atoll and Upolu island, Samoa

Ant community composition

The composition and abundance of ants in communities invaded by *A. gracilipes* was significantly different from uninvaded islands on Nukunonu Atoll (ANOSIM Global $R = 0.934$, $P = 0.001$). Additionally, for *A. gracilipes*-invaded islands only, we found a significant difference in the composition of the local ant communities between the two *A. gracilipes* haplotypes (A[+C] and D) (ANOSIM Global $R = 0.046$, $P = 0.036$). The relatively high abundance of ground-foraging *A. gracilipes* on island with haplotype D relative to A, was the likely driver for these differences. The mean relative abundance of *A. gracilipes* from pitfall trap was on average, an order of magnitude higher on islands harbouring *A. gracilipes* haplotype D, and of the 16 ant species recorded from pitfall traps over all seven islands, 50% were entirely absent from islands with haplotype D: Nukunonu, Motuhaga and Te Puka I Mua (Table 1).

Effect of floristic composition on ant fauna

Coconut palms, *Cocos nucifera*, were the most abundant species on all islands surveyed. There was no significant difference in floristic composition between *A. gracilipes*-invaded islands and uninvaded islands (ANOSIM Global $R = -0.089$, $P = 0.757$), indicating that despite the effect of *A. gracilipes* on ant communities here, vegetation was probably not the driver for this difference. However, the floristic composition differed significantly between islands placed in groupings of *A. gracilipes* haplotype (ANOSIM Global $R = 0.517$, $P = 0.002$). This difference in vegetation community may be sufficient to alter ant communities. However, just as plausible is the effect of a new haplotype on one side of the atoll near the primary port and village.

Discussion

Our results support the hypothesis that repeated introductions of *A. gracilipes* have resulted in two behaviourally, genetically, and spatially distinct populations on Tokelau, and only one of these populations has become an abundant pest. This conclusion corresponds with verbal descriptions by local residents of recently problematic populations of yellow crazy ants in high numbers spreading from the port area of Nukunonu, and failed attempts to slow its spread by burning trees (Lester and Tavite 2004). The *A. gracilipes*

Table 1 Mean number of ants in pitfall traps (±SE) on islands grouped by *A. gracilipes* haplotype, and uninvaded islands on Nukunonu Atoll, Tokelau, in June–July 2005

Species	*A. gracilipes*-invaded islands		Uninvaded islands
	Haplotype A ($n = 40$; 4 islands)	Haplotype D ($n = 30$; 3 islands)	($n = 20$; 2 islands)
Anoplolepis gracilipes	34.83 ± 8.31	242.97 ± 112.61	–
Pheidole sexspinosa	0.15 ± 0.08	0.03 ± 0.03	–
Pheidole oceanica	0.83 ± 0.25	–	0.35± 0.17
Pheidole umbonata	0.48 ± 0.30	0.07 ± 0.07	0.05 ± 0.05
Pheidole fervens	0.78 ± 0.30	1.37 ± 0.65	0.35 ± 0.21
Tetramorium simillimum	0.35 ± 0.18	1.47 ± 1.04	0.05 ± 0.05
Tetramorium bicarinatum	–	–	1.25 ± 0.82
Tetramorium tonganum	0.20 ± 0.20	–	0.20 ± 0.14
Tetramorium lanuginosum	0.80 ± 0.42	4.27 ± 1.29	0.05 ± 0.05
Monomorium floricola	0.05 ± 0.03	0.13 ± 0.08	–
Monomorium minutum	0.03 ± 0.03	–	–
Strumigenys godeffroyi	0.03 ± 0.03	–	–
Pyramica membranifera	0.03 ± 0.03	–	–
Paratrechina vaga	0.10 ± 0.05	–	0.05 ± 0.05
Rogeria stigmatica	0.05 ± 0.03	–	0.05 ± 0.05
Anochetus graeffei	0.08 ± 0.06	–	0.15 ± 0.08
Cardiocondyla nuda	0.03 ± 0.03	0.20 ± 0.17	–
Within-island species richness	7.75 ± 0.75	4.67 ± 0.88	6.0 ± 4.0

Haplotype A islands are Lalo, Te Fala, Pukapuka and Tokelau Island; haplotype D islands include Nukunonu, Motuhaga and Te Puka I Mua. Each island had ten pitfall traps population present on the inhabited islands (Nukunonu, Motuhaga and Te Puka I Mua; see Fig. 1) is composed of haplotype D only (hereafter referred to as 'haplotype-D islands'), whereas haplotypes A and C are both present on Tokelau island, Pukapuka, Te Fala and Lalo (hereafter referred to as 'haplotype-A islands'). Furthermore, populations of *A. gracilipes* on haplotype-D islands are in higher relative abundance and harbour 50% fewer other ant species than those on haplotype-A islands. Haplotype D was not found in the few samples analysed from Samoa, whereas haplotypes A and B were. This result is most likely due to the relatively small sample size from Samoa, however, that two haplotypes were found in such a small sample size suggests that haplotype variation on Upolu island is probably high. The logical conclusion from these results is that *A. gracilipes* displaying haplotype D was introduced from a source population not in Samoa, but further analysis would confirm this conjecture.

Given the relatively recent introduction of *A. gracilipes* to Tokelau (e.g. $<5,000$ years ago), the haplotype diversity present there would most likely not have evolved in situ. Mutation rates for animal mtDNA are commonly cited as 2–2.5% (Brown et al. 1979; Brower 1994). At this rate, in situ changes of *A. gracilipes* mtDNA on Tokelau are highly unlikely. Thus, the presence of two distinct haplotype groupings within Nukunonu Atoll in Tokelau is indicative of at least two separate invasion events. Lester and Tavite (2004) described this scenario based on a sudden increase in the abundance of *A. gracilipes* on some islands on Nukunonu and Fakaofo Atolls, and postulated a more recent invasion originating from a different source population from that of an earlier invasion. Given that we did not find haplotype D in Samoa and that the primary invasion pathway originates in Samoa (ship that services the atolls fortnightly is from Samoa, and there is no airport), the pathway for the 'new' invasion is difficult to identify. Yet, haplotype D is also on the inhabited island on Fakaofo atoll (Fenua Fala), where densities of *A. gracilipes* are extremely high (K.L. Abbott personal observation), so propagules responsible for the 'new' invasion might have been deposited on both atolls from a similar source. One plausible explanation is that ants with haplotype D were transported to Tokelau on a vessel that visited the atolls once or twice. An expanded genetic survey of the Pacific region would help identify a possible source population. Austin et al. (2005) have recently documented the presence of the pestiferous subterranean termite *Reticulitermes flavipes* (Kollar) in South America and that introductions there share identical genetic haplotypes from both the United States and Europe (France and Germany). Using mitochondrial DNA as a tool to determine population history may also hold the key to helping identify high-risk invasion pathways and the origins of invasive species (Ballard and Rand 2005; Sommer 2005).

Ours is the first work on *A. gracilipes* to identify haplotype differences associated with a particular invasion. Intraspecific aggression has been reported between workers in and between supercolonies on Christmas Island previously (Abbott 2005), leading us to suspect that small scale genetic variation within this species is widespread and may contribute to variation in colony structure and organisation. That Tsutsui et al. (2001) were unable to discriminate between introduced populations of Argentine ants using the mtDNA marker cytochrome *b*, might have been a function of their sampling regime and analysis (S. Corin, unpublished data). Their use of maximum likelihood phylogenetic trees (using TREE-PUZZLE; Strimmer and von Haeseler 1996), most often using information from one individual ant per population, may not have been sensitive enough to reveal differences within introduced populations. They sequenced 27 individual ants from 23 populations from Europe, Australia, north and South America, and within California. Here we sequenced 80 individual ants from 13 populations of *A. gracilipes* from Tokelau and Samoa. Alternatively, it is possible that the introduced populations from which they sequenced cytochrome *b* were actually derived from the same source population. This possibility would be highly unlikely however.

It has been hypothesised that social insects are more successful in their introduced ranges due to decreased genetic variation at loci associated with kin recognition (either a bottleneck or genetic 'cleansing') resulting in low levels of intraspecific aggression hence energy to spend on foraging (Suarez et al. 1999; Tsutsui et al. 2000; Giraud et al. 2002). This is a plausible explanation for the differences in aggression observed between the two haplotypes on Tokelau. Within haplotypes intraspecific aggression was non-existent. Between haplotypes A and D, ants fought consistently, indicating lack of recognition as kin. However, the dearth of information about colony structure and behaviour of *A. gracilipes* in its native range means that this hypothesis is still open to rigorous analysis.

Differential impacts of A. gracilipes haplotype?

That *A. gracilipes* has been present on an isolated oceanic atoll such as Nukunonu Atoll, for at least 60 years is not surprising, given its propensity for human-mediated dispersal and the fact that humans have been traversing the Pacific region for over 2000 years (Gibbons 2001). However, the recent increase in its abundance on haplotype-D islands has led to social, environmental and health problems. The Polynesian lifestyle is an outdoor one, where most activities, including eating, are conducted on the ground. Human social interactions on Tokelau shifted to inside houses in areas where *A. gracilipes* numbers were high outside, and children and older people have reported irritation by foraging ants while sleeping (Lester and Tavite 2004). If this haplotype is indeed a more invasive, or aggressive type, then its presence on other Pacific islands could be detrimental.

Fifty percent of other ant species present on haplotype-A islands (Lalo, Te Fala, Pukapuka and Tokelau Island) were missing from haplotype-D islands (Nukunonu, Motuhaga and Te Puka I Mua). Their absence from the ant fauna where *A. gracilipes* numbers are high is consistent with observations on other islands where *A. gracilipes* extirpates ant species in high-density supercolonies (Abbott 2004). As *A. gracilipes* density increased across supercolony boundaries on Christmas Island, Indian Ocean, other ant species richness decreased (Abbott 2006). In addition, in Hawaii, *A. gracilipes* actively defended inflorescences, from which they were sequestering carbohydrate-rich nectar, from a simulated competitor (Lach 2005). The eight ant species that are deleted from the high-density *A. gracilipes* islands cover a range of sizes, from the tiny *Monomorium minutula* (head width 0.3 mm; Sarty 2005) to the large ambush predator *Anochetus graffaei* (head width 0.9 mm; Sarty 2005). In India, *A. gracilipes* was noted to have eliminated two large dominant predatory ants *Oecophylla smaragdina* (Fabricus) and *Leptogenys processionalis* (Jerdon) (Veeresh and Gubbaiah 1984), while on Christmas Island it typically coexisted with only two of the approximately 40 resident ant species: *Paratrechina longicornis* (Latreille), and *Paratrechina minutum* (Forel).

The unethical practice of transplanting, moving or introducing an invasive species into a previously uninvaded habitat renders it difficult to tease out the relative importance of factors responsible for direct impacts, other than in small-scale experiments. As a result, we cannot decisively conclude that the different *A. gracilipes* haplotypes observed within the communities are the leading cause of differential impacts by *A. gracilipes* on ant communities. Indeed, the analysis of the plant community demonstrated that there were differences in the floristic composition between islands inhabited by each *A. gracilipes* haplotype. The less common plant species are intrinsically linked to humans and could not be separated from a factor that took into account disturbance or habitation. Similarly, two of the three haplotype-D islands are inhabited, albeit by less than 450 people. But the habitation by humans in combination with food plants and disturbance on these islands is a feasible alternative hypothesis for the lower ant species richness observed there.

Over time, it is likely that *A. gracilipes* with haplotype D will be transferred to islands occupied by *A. gracilipes* haplotype A, which, if the transfer is detected, will provide a unique opportunity to examine how the ant communities change with invasion of a new haplotype. It may also offer information about haplotype-specific phenotypes that results in the numerical superiority of *A. gracilipes* haplotype D. That innate characteristics of *A. gracilipes*, as opposed to attributes of the recipient environment, are responsible for their ecological success, is a hypothesis contrary to that developed for Argentine ants, *Linepithema humile*, and the red imported fire ant, *Solenopsis invicta* (e.g. Holway et al. 2002; Porter et al. 1997) and warrants attention in the pursuit of explaining the success of invasive ants. Further research is also necessary to elucidate more specific phenotypic and behavioural differences between haplotypes, but the result of this study allowed us to gain insight into the species-specific role of *A. gracilipes* in the modification of ant community structure, for theoretical as well as applied ecological advancement.

Previously, biosecurity authorities have been much less concerned over the introduction of a species already existing within a country's border than an introduction of an entirely new species. Our research suggests that these authorities should be concerned over genetic variation at the population level, rather than simply its Latin identity, as in the case of *Phragmites* in the United States. Should such genetic diversity and its differential effects on community be typical among invasive species, it may help explain the spatial and temporal variation in invader abundance and effects elsewhere (Sakai et al. 2001; Simberloff and Gibbons 2004).

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