Population genetics and history of the introduced fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in Australia

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Abstract
The red imported fire ant, *Solenopsis invicta*, a damaging invasive pest, was discovered in February 2001 in Brisbane, Australia at two sites, Fisherman Islands and suburban Richlands-Wacol. Using four microsatellite loci and the protein marker *Gp-9*, we compared the two infestations with each other, and with potential source populations in North and South America to better understand the history of their introduction to Brisbane. Based on an analysis of molecular variance, as well as a maximum likelihood tree of colonies from the two Australian sites, we found that the two sites were genetically distinct and were almost certainly introduced separately. All of the colonies at Fisherman Islands were monogynous, headed by a single queen, while the Richlands-Wacol site had a mixture of single queen monogynous and multiple-queen polygynous colonies. However, the monogynous and polygynous colonies at the Richlands-Wacol site were not genetically distinct from each other, and probably constitute a single, mixed introduction. Based on allele frequencies at the microsatellite loci, and *Gp-9*, both Australian infestations were more similar to North American populations than to South American, though the Fisherman Islands infestation was intermediate, making it difficult to assign. Thus, there has been one introduction from either a North or South American monogynous population at Fisherman Islands, and one introduction from a mixed monogynous/polygynous North American population at Richlands-Wacol. These findings have implications for the control of the current infestations, as well as for the quarantine regulations necessary to prevent additional introductions to Australia.

Key words *Gp-9*, invasive species, microsatellites, red imported fire ant.

INTRODUCTION

The red imported fire ant, *Solenopsis invicta*, is native to South America (Taber 2000), but has become a potential major exotic pest worldwide, as shown by expansion in the West Indies (Davis *et al.* 2001), New Zealand (Pascoe 2002), and Puerto Rico (Callcott & Collins 1996). It has been particularly destructive in North America. Since its introduction to North America in the 1930s (Tschinkel 1998), it has invaded over 120 million ha, and, in Texas alone, costs annually over US$1.2 billion in damage and for control (Lard *et al.* 2002). The invasion of *S. invicta* has devastating consequences for native invertebrate (Porter & Savignano 1990; Hu & Frank 1996; Forys *et al.* 2001; Stiles & Jones 2001; Wojcik *et al.* 2001) and vertebrate (Lofgren *et al.* 1975; Allen *et al.* 1995, 1997a,b, 2001a,b; Lechner & Ribble 1996) communities.

In February 2001, *S. invicta* was found in Brisbane, Australia at two sites separated by approximately 30 km: Fisherman Islands, a port facility located at the mouth of the Brisbane River, and suburban Richlands-Wacol, in south-west Brisbane (Nattrass & Vanderwoude 2001; McCubbin & Weiser 2002; Moloney & Vanderwoude 2002; Solley *et al.* 2002). The fire ant was estimated to have arrived only 3–5 years earlier, but had already occupied more than 40 000 ha (Vanderwoude *et al.* 2004). Based on the minimum and maximum climatic conditions endured by the fire ant, Vanderwoude *et al.* (2004) believed that it has the potential to invade and inhabit more than 95% of urban Australia. To prevent this outcome, an extensive 5-year program of control and eradication is underway (Vanderwoude *et al.* 2004). As part of such a program, as well as continuing efforts to prevent additional introductions, it is important to gain an understanding of the history of the fire ant's introduction to the Brisbane area.

Among the questions to be answered are whether multiple introductions have occurred and how they correspond to the known infestations. The Fisherman Islands and Richlands-Wacol infestations may have resulted from a single introduction that established itself at multiple sites, or there could have been separate introductions at each site. There may have even been multiple introductions at each site. The number of intro-

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ductions and how they have become established at different sites will have implications for quarantine measures, as well as for an understanding of the spread of the current infestation and how best to control it.

Knowledge of the epidemiology of the current infestations after they have become established is also important. In particular, there are two distinct social forms of *S. invicta* which have different population densities and modes of colony foundation that can affect their spread. The monogynous form has a single queen and effectively excludes foreign queens and workers from the colony. The polygynous form has many queens, and lacks effective colony discrimination (Glancey *et al.* 1973; Fletcher *et al.* 1980; Bhatkar & Vinson 1987; Tschinkel 1998). This allows polygynous colonies to exist at much higher densities than monogynous colonies (Macom & Porter 1996), increasing their destructive potential. However, the monogynous form poses a greater challenge to effective monitoring because its colonies are founded by queens after a dispersal flight that can carry them several kilometres away (Markin *et al.* 1971; Tschinkel 1998). In contrast, polygynous colonies are dispersed through colony fission over short distances and adoption of new queens by other colonies (Glancey & Lofgren 1988). Previous work has suggested that the Fisherman Islands site is solely monogynous and that the Richlands-Wacol site has a mixture of monogynous and polygynous colonies.

A codominant mendelian locus, *General protein-9* (*Gp-9*), is strongly correlated with social form in *S. invicta* (Keller & Ross 1999), and can be used to characterise the social form of the Australian colonies. In polygynous colonies all of the queens are *Gp-9^Bb* because *Gp-9^BB* queens are executed by the workers, and *Gp-9^bb* is lethal (Ross *et al.* 1999). In monogynous colonies, the queen is always *Gp-9^BB* (Goodisman *et al.* 2000). Thus all of her offspring, the workers and males, only have the B allele (mating between monogynous queens and polygynous males is believed to be extremely rare). There is also a cryptic b' allele in South American populations that functions as a b allele, but whose protein product behaves electrophoretically on starch gels as a B allele (Krieger & Ross 2002).

The source point for the incursion in Australia is also of quarantine interest. The fire ant has proven difficult to eradicate wherever it has established itself, and it is fortunate that it was discovered soon enough after its introduction in Brisbane that eradication is still possible. But the best hope for an *S. invicta* free Australia in the long term is effective quarantine. This will necessitate identifying the most likely sources of introduction.

In this study, we used highly polymorphic microsatellites as well as the *Gp-9* locus to characterise the population genetic structure of the Australian fire ant infestation and to confirm which social forms occur at each site. By characterising any differences between the two infestations, between the different social forms, and between the Australian infestations and North and South American populations, we determined how many introductions occurred, which social forms were introduced, and from where they were introduced.

MATERIALS AND METHODS

Fire ant collection

Between 7 October 2001 and 14 October 2001, we collected workers and males from 40 colonies at 20 sites in the Richlands-Wacol infestation, and 7 colonies at 4 sites in the Fisherman Islands infestation. We subsequently collected additional males from 13 colonies in the Richlands-Wacol infestation and 5 colonies in the Fisherman Islands infestation. All sampled colonies were putatively determined to be either monogynous or polygynous based on the size of the workers and the numbers of queens detected. The Queensland Department of Primary Industries also provided a single ant from each of 19 North American fire ant colonies (Florida, Georgia, Texas) and 7 South American colonies (Argentina) for comparison.

Extraction of genomic DNA

Genomic DNA was extracted using a commercially available salt precipitation protocol (Puregene DNA isolation kit, Gentra Systems). A minimum of 10 individuals, including both major and minor workers and males, were extracted from each nest. We used the whole ant for each extraction and the DNA was resuspended in 50 μL of water for minor workers, 75 μL for major workers, and 100 μL for males.

General protein-9 genotyping

We genotyped 223 workers and 168 males at the *Gp-9* locus using previously published polymerase chain reaction (PCR) primers and protocols (Krieger & Ross 2002). Following PCR amplification, the products were subjected to a restriction fragment length polymorphism (RFLP) assay designed by Krieger (pers. comm. 2001) to distinguish between the *Gp-9^bb*, *Gp-9^Bb*, *Gp-9^BB* genotypes. We digested 5 μL of each sample with 1 μL of the restriction enzyme *BsaAI* (5 units per μL; New England Biolabs) overnight at 37°C in a 25 μL reaction mixture. Following complete digestion, RFLPs were visualised on 4% agarose gels, stained with ethidium bromide, and photographed under UV light.

The RFLP assay was unable to distinguish between the b and b' alleles and we sequenced *Gp-9* from seven diploid males which were scored as having the *Gp-9^Bb* genotype to determine whether any had the b' allele. Males are normally haploid, arising from unfertilised eggs, but diploid males can occur when fertilised, female-destined eggs are homozygous at the sex determining locus and develop as males instead. Diploid males frequently occur in populations that have reduced heterozygosity as a result of a bottleneck, and they are a common occurrence in North American polygynous colonies. Sequences were obtained using the DYEnamic ET sequencing kit (Amersham Pharmacia) and visualised on an ABI 377 automated sequencer (Applied Biosystems).

Microsatellite PCR amplification

We genotyped the workers and males at four microsatellite loci: *sol 6*, *sol 11*, *sol 18*, and *sol 20* (Krieger & Keller 1997).

Microsatellite loci were amplified via PCR using 10 µL reactions containing 1 µL of PCR buffer (Promega 10x buffer containing 15 mM MgCl₂), 1 µL of 2.5 mM dNTPs (Amersham Pharmacia), 0.5 µL of 10 µM fluorescently labelled forward primer, 0.5 µL of 10 µM reverse primer, 0.1 µL of Taq polymerase (5 units per µL), and 2 µL of template DNA (Krieger & Keller 1997). Reactions were cycled through an initial denaturation at 92°C (2 min), followed by 35 cycles at 92°C (45 s), 55°C (45 s), and 72°C (1 min), and a final elongation step of 72°C (5 min). We visualised the PCR products on 5% denaturing polyacrylamide gels using the Gel-Scan 2000 electrophoresis system (Corbett Research).

Calculation of colony relatedness

We calculated relatedness among the workers in each colony based on their microsatellite genotypes according to the method of Queller and Goodnight (1989) using the computer program Relatedness 5.0 (Goodnight & Queller 1999). We estimated population allele frequencies in the Fisherman Islands and Richlands-Wacol populations separately and estimated standard errors by jackknifing over loci for colony estimates and over colonies for population estimates. We tested for differences in relatedness between populations and social forms using a one-way ANOVA and Fisher's Least Significant Difference (LSD) for $post\ hoc$ tests.

Population structure and the number of introductions

To determine whether the Richlands-Wacol monogynous, polygynous, and Fisherman Islands colonies were introduced separately, we examined the partitioning of genetic variance, estimated from microsatellite allele frequencies, within and between infestation sites and social types using the Analysis of Molecular Variance (AMOVA) framework (Excoffier et al. 1992) implemented in the computer program Arlequin (Schneider et al. 2000). Significant partitioning of a large amount of the genetic variance between infestation sites or social forms would indicate differentiation, supporting the inference of multiple introductions.

Because the polygynous social form is only found at the Richlands-Wacol site, we also examined the partitioning of genetic variance between sites using only the monogynous colonies, and the partitioning of variance between social forms using only Richlands-Wacol colonies. The results did not change, and we only report the results for all colonies here.

To further assess the differentiation between infestation sites and social forms, we created both neighbour-joining and maximum likelihood trees of the colonies. We used the computer program Distance to compute Nei's D as a measure of genetic distance between colonies, Neighbour to create the neighbour-joining tree of colonies based on the genetic distances and ContML to create the maximum likelihood tree of colonies. These programs are part of the Phylip package of computer programs (Felsenstein 1993). The two trees were quantitatively the same and we only report the maximum likelihood tree.

Origins of the Australian invasion

To assess whether fire ants were introduced from North or South America, we also created neighbor-joining and maximum likelihood trees joining the Richlands-Wacol monogynous population, the Richlands-Wacol polygynous population, the Fisherman Islands monogynous population, the North American and South American samples provided by the Queensland Department of Primary Industries, and nine North American populations from Georgia and Louisiana whose allele frequencies were previously published (Ross et al. 1999). The two trees were quantitatively the same and we only report the maximum likelihood tree.

Finally, we compared the microsatellite allele frequencies in the Australian fire ants with the average allele frequencies in the nine North American populations (Ross et al. 1999) to determine whether the Australian and North American populations have similar subsets of the known alleles from South America (L. Keller pers. comm. 2002).

RESULTS

Social form

All of the workers and males at the Fisherman Islands site had $Gp-9^{BB}$ and $Gp-9^B$ genotypes, respectively (Table 1). The workers and males from the putatively monogynous colonies at the Richlands-Wacol site were also exclusively $Gp-9^{BB}$ and $Gp-9^B$, respectively. The workers and males from the putatively polygynous colonies at the Richlands-Wacol site had a mixture of genotypes that included the $Gp-9^b$ allele (Table 1). This allele is only found in polygynous colonies. Thus the Fisherman Islands site is entirely monogynous, while the Richlands-Wacol site is a mixture of monogynous and polygynous colonies.

None of the seven diploid males with $Gp-9^{bb}$ genotypes had the cryptic b' allele. This allele is found in some South American populations (Mescher et al. 2003), at a frequency of about 30%, but is not known in any North American populations. All of the sequenced males were from the Richlands-Wacol population and this result suggests that the Richlands-Wacol population is derived from North America. However, the 95% confidence interval for the frequency of the b' allele in the Richlands-Wacol population includes 0% to 41%, so a South American origin cannot be excluded on this evidence.

We also found that many of the males from the polygynous colonies were $Gp-9^{bb}$ heterozygotes, indicating that they were diploid. Additional diploid males with $Gp-9^{Bb}$ and $Gp-9^{BB}$ genotypes were detected because they were heterozygous at one or more of the microsatellite loci. Diploid males are a frequent occurrence in North American polygynous colonies, but not in monogynous colonies or in South America. This suggests that the polygynous colonies were introduced from North America. However, an alternative explanation is that they were introduced from South America and experienced a genetic bottleneck that reduced heterozygosity at the sex determining locus leading to increased production of diploid males.

Table 1 Frequency of *Gp-9* genotypes in *Solenopsis invicta* workers and males, within each social type

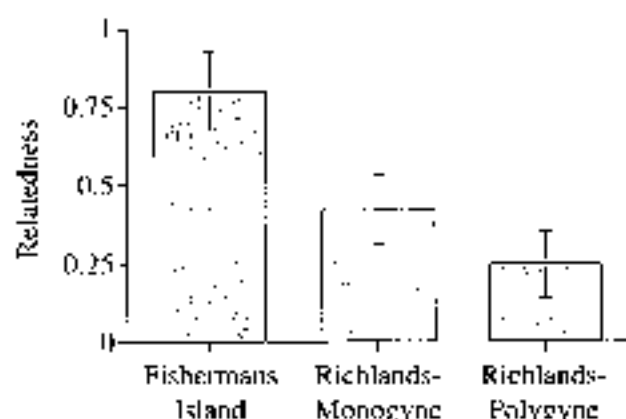| Genotype	Workers			Males		
	Fishermans Islands	Richlands-Wacol		Fisherman Islands	Richlands-Wacol	
	Monogynous (n = 34)	Monogynous (n = 95)	Polygynous (n = 94)	Monogynous (n = 56)	Monogynous (n = 65)	Polygynous (n = 47)
BB	1.0000	1.0000	0.3293	0.0000	0.0000	0.0551
Bb	0.0000	0.0000	0.6556	0.0000	0.0000	0.5957
bb	0.0000	0.0000	0.0146	0.0000	0.0000	0.1489
B				1.0000	1.0000	0.0638
b				0.0000	0.0000	0.1065

Fig. 1. Within-nest relatedness (±95% confidence interval) in *Solenopsis invicta* determined by the Fisherman Islands site (all colonies monogynous) and for the monogynous and polygynous colonies separately at the Richlands-Wacol site, Brisbane.

Relatedness should be lower in polygynous colonies than in monogynous colonies because polygynous colonies have many queens. We found that relatedness in the putatively polygynous colonies was significantly lower than in either the monogynous Fisherman Islands, or monogynous Richlands-Wacol colonies as expected (Fig. 1). However, while workers in the Fisherman Islands colonies were approximately related as full sisters (r = 0.75), as would be expected under a single, once-mated queen, relatedness in the Richlands-Wacol monogynous colonies was significantly lower than in Fisherman Islands and was not representative of a single queen as expected.

Number of introductions

The AMOVA analysis revealed that 26% of the genetic variance in the Australian population was between the Richlands-Wacol and Fisherman Islands sites (Table 2), indicating that they are genetically distinct from each other and probably constitute separate introductions to Australia. Only 1% of the genetic variance was found between the polygynous and monogynous colonies in the Richlands-Wacol population (Table 2). This was not significantly different from zero, and they most likely constitute a single, mixed introduction from a locale where both social forms occur together.

Table 2 Results of the AMOVA analysis partitioning the genetic variation in *Solenopsis invicta* among and within collection sites, populations (Richlands-Wacol or Fisherman Islands), and social forms (monogynous or polygynous). The P-value estimates the probability of a more extreme variance component and φ-statistic by chance alone

Variance component	Variance	% total	φ-statistic	P
Variance partitioned by collection site and social type				
Between social types	0.01364	1.05	0.011	0.23
Within social type	0.23040	19.15	0.194	<0.00008
Within collection sites	0.95533	79.77	0.203	<0.00001
Variance partitioned by collection site and population				
Between populations	0.35765	26.15	0.26152	0.0096
Within populations	0.11805	7.97	0.10800	<0.00001
Within collection sites	0.97800	65.88	0.54154	<0.00001

The maximum likelihood and neighbour-joining trees of the Australian colonies agree with the results of the AMOVA. The Fisherman Islands colonies cluster into a single group, and the monogynous and polygynous colonies in the Richlands-Wacol area all cluster together (Fig 2). Thus, the two populations are distinct from each other and were probably introduced separately. The longest distance in the dendrogram is that from our South American sample to the rest, and according to the 'approximate confidence limits' provided by CONTML it is significantly longer than any other branch.

Source populations

The maximum likelihood tree of Australian, North American, and South American populations show the South American sample to be the most divergent, and clearly distinct from the Australian and North American populations (Fig. 3). The Richlands-Wacol population clusters closely with the North American populations. The Fisherman Islands population is more similar to the North American populations than to the South American sample, but it is intermediate, making it difficult to determine its origin with certainty.

Although the distance from the South American sample to the rest of the tree is significantly the largest distance, it may have been inflated by the effects of small sample size (Takezaki & Nei 1996). However, as for studies on the
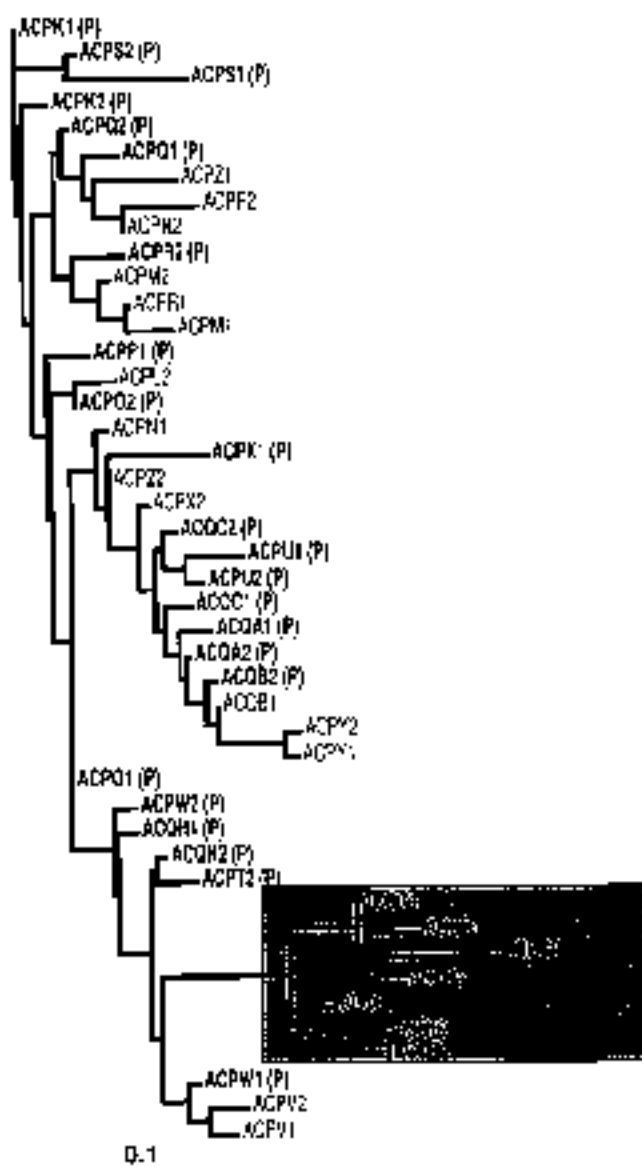
ACPK1 (P)
ACPS2 (P)
ACPS1 (P)
ACPK2 (P)
ACPQ2 (P)
ACPQ1 (P)
ACPZ1
ACPF2
ACPN2
ACPH2 (P)
ACPW2
ACFR1
ACPM1
ACPP1 (P)
ACPL2
ACPO2 (P)
ACPN1
ACPK1 (P)
ACPZ2
ACPX2
ACQC2 (P)
ACPU1 (P)
ACPU2 (P)
ACQC1 (P)
ACQA1 (P)
ACQA2 (P)
ACQB2 (P)
ACQB1
ACPV2
ACPV1
ACPO1 (P)
ACPW2 (P)
ACQH1 (P)
ACQH2 (P)
ACPT2 (P)

ACPW1 (P)
ACPU2
ACPV1

0.1

Fig. 2. Maximum likelihood tree of all *Solenopsis invicta* colonies sampled from Australia. Monogynous colonies are shown in grey and polygynous ones in black. The boxed colonies were those collected on Fisherman Islands. The scale gives expected accumulated variance (Felsenstein 1993).

North America
(Louisiana)
Fisherman Islands
(Georgia)
Richlands-Waco
(Monogynous)
(Polygynous)
North American Sample

0.1

South American Sample

Fig. 3. Unrooted maximum likelihood tree of the three Australian populations of *Solenopsis invicta* together with published North American populations and small samples from North and South America.

Argentine ant, *Linepithema humile* Mayr (Tsutsui *et al.* 2001), we can also use the fact that introduced populations will have subsets of the alleles of the source areas. The Australian and North American populations had strikingly similar subsets of the known South American alleles at the four microsatellite loci (Table 3). There were a total of 58 known South American alleles at the four loci and 25 of these were also known from North America. Even though less than 50% of the known South American alleles were found in North America 17 of the 21 alleles in the Australian populations were also in the North American subset of alleles.
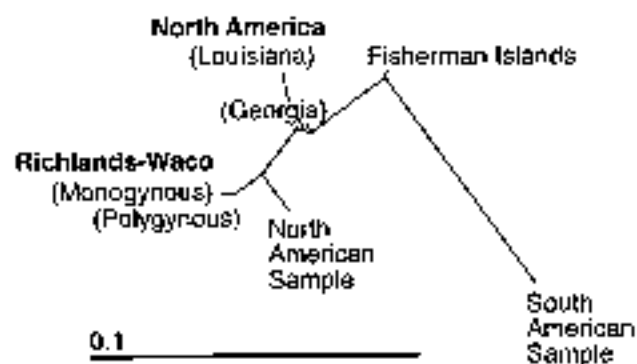
DISCUSSION

Our results indicate that the two infestations at Fisherman Islands and Richlands-Waco1 are genetically distinct and have most probably been introduced separately. The Richlands-Waco1 site contains both monogynous and polygynous colonies, but there is no evidence that these are genetically distinct from each other at the microsatellite loci even though they are distinctly different at the *Gp-9* locus. This is most likely because the Richlands-Waco1 infestation was introduced from a mixed monogynous/polygynous population. Migration from monogynous populations into adjacent polygynous populations in North America has been shown to reduce genetic differences at neutral loci, such as microsatellites (Ross & Shoemaker 1993), while not affecting differences at *Gp-9* which are under selection (Gotzek *et al.* 2000). Thus, there has been one introduction from a pure monogynous population at Fisherman Islands, and another introduction from a mixed monogynous/polygynous population in Richlands-Waco1.

North America is the most likely source of both infestations. The evidence is particularly strong for the Richlands-Waco1 infestation. The polygynous colonies at Richlands-Waco1 have a high proportion of diploid males, a characteristic of North American polygynous colonies, but not of South American ones. Polygynous colonies at the Richlands-Waco1 infestation also appear to lack the b² allele, which is found in some South American populations with a frequency of about 30% but is absent in North American populations. Finally, the Richlands-Waco1 infestation was highly genetically divergent from the South American sample, but extremely similar to North American populations as seen in the maximum likelihood tree. The Fisherman Islands infestation was also more similar to the North American populations than to the South American sample, but it was intermediate, making it difficult to assign. Mitochondrial DNA sequence information has been shown for Argentine ants to add to the picture obtainable from microsatellites (Tsutsui *et al.* 2001), and is being collected for

Table 3 Allele frequencies at four microsatellite loci in *Solenopsis invicta* in the Richlands-Wacol polygynous (RW-P), Richlands-Wacol monogynous (RW-M), and Fisherman Islands (FI) populations compared to the known alleles from South America (Argentina, L. Keller pers. comm. 2002), and the average population allele frequencies from nine previously published North American populations (Ross *et al.* 1999). Alleles in parentheses are not known from South America but were observed in either North American or Australian populations

Locus	Known South American alleles	RW-P (n = 23)	RW-M (n = 16)	FI (n = 7)	North America
Sol-6	87				
	95				0.001
	97				
	107				
	109	0.017		0.645	0.005
	111				0.002
	113	0.570	0.632	0.364	0.692
	115	0.406	0.364		0.270
	117				
	119				
	121	0.007	0.004		
	123				0.001
	125				
	127				
	129				
	131				
	133				
Sol-11	143	0.086	0.063	0.152	0.419
	145	0.091	0.142		0.060
	147	0.540	0.506		0.099
	149	0.066			0.001
	151	0.266	0.295	0.773	0.305
	153				
	155			0.074	0.209
	157				
	159				
	161				
	163				
	165			0.015	
	167				
	169				
	(171)				0.001
	177				
Sol-18	117				
	123				
	125	0.366	0.449	0.734	0.658
	127	0.472	0.414	0.266	0.141
	129	0.156	0.101		0.300
	131				
	135				
	(139)	0.006	0.036		
Sol-20	(115)				0.300
	120				
	122				
	124	0.374	0.499		0.146
	126	0.103	0.038	0.544	0.412
	128	0.220	0.161	0.426	0.168
	129				
	130			0.029	0.006
	131				
	132				0.001
	134				
	136				0.002
	138				
	140				
	142				
	144				0.155
	146				0.001
	148	0.007			
	150				0.008
	152	0.297	0.301		0.100

Australian *S. invicta* by Prof. Jane Hughes (Griffith University, Brisbane).

The agreement between the Australian and North American allele size distributions indicates an origin of both invasive populations from North America. However, it is possible that the alleles found in North America were those of high frequency in South America and that a bottleneck in an Australian population originating from South America might lead to the same alleles entering Australia independently of the situation in North America. Populations of *I. invicta* outside the native range appear to be due to a single original introduction and this is reflected by their having similar subsets of the alleles to those seen in the native range (Tsutsui *et al.* 2001).

Our results have several implications for the current plan to eradicate *S. invicta* from the Brisbane area and to prevent additional introductions to Australia. First of all, it is important that the two infestations are not the result of dispersal to multiple sites by a single infestation. Such a pattern of dispersal would indicate a high probability of other large undiscovered infestations in the south-east Queensland area which would jeopardise the current eradication program and dramatically increase the area to be monitored for successful eradication. However, it also suggests that additional quarantine effort may be necessary if the risk of future introductions is to be minimised, given that the current level has clearly been inadequate.

North America was almost certainly the source of one of the infestations and may also be the source of the other, suggesting that North America may be a more likely source than South America. This may be because of the greater volume of goods arriving from North America relative to South America (Anonymous 2003; Australian Bureau of Statistics 2003), or it could be because North American *S. invicta* occur in higher densities (Porter *et al.* 1997), making their accidental inclusion in shipped goods more likely (however, we acknowledge that there is no evidence that *S. invicta* were actually transported with shipped goods). Another possible explanation is that *S. invicta* arrived in Brisbane from another country or location where they have established but have not yet been detected.

These infestations in Brisbane have shown that, once introduced, *S. invicta* can remain undetected for years, quietly establishing themselves at high densities over large areas. Given the difficulty in eradicating fire ants once they are established, effective quarantine is the best means of minimising the risk of their establishment in Australia in the long term. However, effective quarantine for this and other pests should be supported by comprehensive surveillance programs, for example, bait and trap measures such as introduced at New Zealand entry points, to ensure new incursions are detected before economic and technical impediments (size of infested areas) reduce options for management or eradication of exotic pests.

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