

# The distribution and evolutionary history of *Wolbachia* infection in native and introduced populations of the invasive argentine ant (*Linepithema humile*)

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## Abstract

*Wolbachia pipientis* is a maternally transmitted bacterium that often alters the life history of its insect host to maximize transmission to subsequent generations. Here we report on the frequency and distribution of *Wolbachia* infection in a widespread invasive species, the Argentine ant (*Linepithema humile*). We screened 1175 individual Argentine ants from 89 nests on five continents and several islands, including numerous locations in both the native (South American) and introduced ranges. We detected *Wolbachia* in four of 11 native populations, but only one of 21 introduced populations was infected. In the Argentine ant's native range, the distribution of *Wolbachia* supergroups A and B was nonoverlapping. By coupling infection frequency data with behaviourally defined colony boundaries, we show that infected and uninfected colonies are often adjacent to one another, supporting the proposition that little female-mediated gene flow occurs among Argentine ant colonies. We also conduct a phylogenetic analysis, and show that the *Wolbachia* infecting both native and introduced populations of Argentine ants belong to two lineages that appear to be specialized on infecting New World ants. One other lineage of *Wolbachia* has undergone frequent, recent episodes of horizontal transmission between distantly related, introduced insect hosts.

**Keywords:** cytoplasmic sex ratio distorters, *ftsZ*, horizontal transmission, selfish elements, social insects, *wsp*

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## Introduction

*Wolbachia pipientis* is an  $\alpha$ -proteobacterium that, like many other cytoplasmic elements, often plays a major role in the evolution of its hosts (Hurst 1993; O'Neill *et al.* 1997; Werren 1997; Stouthamer *et al.* 1999). Because the bacterium is transmitted maternally, it can increase its fitness by inducing life history changes in its hosts that increase the production of daughters by infected females, leading to potential conflicts of interest between parasite and host over the favoured sex ratio. There are numerous examples of host sex-ratio manipulation from various arthropods,

mediated by feminization of males, killing of males and parthenogenesis (reviewed in Hurst 1993; O'Neill *et al.* 1997; Werren 1997; Stouthamer *et al.* 1999). In addition, in some taxa *Wolbachia* infection may cause cytoplasmic incompatibility in matings between infected and uninfected individuals, or between individuals infected with different strains of the bacterium (reviewed in Hurst 1993; O'Neill *et al.* 1997; Werren 1997; Stouthamer *et al.* 1999). In the case of haplodiploid social insects, infection by *Wolbachia* may complicate the sex-ratio conflict further between host and parasite, because there is an inherent pre-existing conflict between queens and workers regarding the optimal sex ratio of reproductive offspring (Trivers & Hare 1976).

*Wolbachia* may also influence a host's evolutionary history, social organization and population structure (O'Neill *et al.* 1997; Werren 1997; Jiggins *et al.* 2000; Jiggins 2002; Wenseleers *et al.* 2002). For example, *Wolbachia*-induced

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incompatibility has promoted speciation in *Nasonia* wasps (Bordenstein *et al.* 2001). Recent work has also implicated *Wolbachia* in the origin of sex-role-reversed swarming in the butterfly *Acraea encedon*, probably in response to the scarcity of males resulting from the effects of the male-killing *Wolbachia* infecting the host females (Jiggins *et al.* 2000; Jiggins 2002). In *Drosophila simulans*, the close association between mitochondrial haplotypes and *Wolbachia* strains suggests that as *Wolbachia* infection spreads through a population it drives the transmission of (maternally inherited) mitochondrial types as well (reviewed in O'Neill *et al.* 1997).

Conversely, the evolutionary history, phylogeography, population structure and social organization of host species may shape the evolution, geographical distribution and phenotypic effects of *Wolbachia* (e.g. Turelli & Hoffman 1995; O'Neill *et al.* 1997; Wenseleers *et al.* 1998). For example, *Wolbachia* should spread more easily in host species that undergo repeated population extinction and recolonization because the associated founder events can allow infection frequencies to exceed unstable levels (Turelli & Hoffman 1995). Hosts that disperse long distances can transmit *Wolbachia* infection more rapidly and across larger geographical areas than locally dispersing hosts (Turelli & Hoffman 1995) and theoretical studies predict that *Wolbachia* infection should spread more slowly in subdivided host populations relative to panmictic ones (Wade & Stevens 1994).

The Argentine ant (*Linepithema humile*) presents a unusual opportunity to examine how host history, dispersal and social organization affect the distribution of *Wolbachia* infection. *L. humile* is a widespread invasive species (Suarez *et al.* 2001) that promotes agricultural damage by tending aphids, scale insects and other Homoptera (Horton 1918; Way 1963; reviewed in Holway *et al.* 2002). Introduced Argentine ants also displace or harm native species (reviewed in Holway *et al.* 2002) and are a major pest in businesses and households (Newell & Barber 1913; Knight & Rust 1990).

In many other host species, historical phylogeography and patterns of range expansion can be difficult to reconstruct. In Argentine ants, however, the recent history of introductions is well known (Suarez *et al.* 2001) and population genetic studies have been performed in both the native and introduced ranges (Suarez *et al.* 1999; Tsutsui *et al.* 2000, 2001, 2003; Tsutsui & Case 2001; Giraud *et al.* 2002). These studies suggest that founder effects, genetic bottlenecks and rapid population expansion have been associated with the introduction and establishment of new populations (Tsutsui *et al.* 2000, 2001; Tsutsui & Case 2001). Consequently, the frequency and distribution of *Wolbachia* infection may differ between the native and introduced ranges of its Argentine ant host. Moreover, to the extent that geographical boundaries and distance constrain maternal gene flow among Argentine ant populations,

*Wolbachia* may be limited to particular regions, or may be distributed in patterns that reflect the host's phylogeographical structure.

Argentine ants also possess various types of local population structure that may further affect the distribution of *Wolbachia* infection. Argentine ant populations typically consists of behaviourally distinct (territorial) colonies, consisting of multiple nest sites that contain workers, brood, and reproductive queens and males. However, the typical size of these social groups differs between the native and introduced ranges. Introduced populations form vast 'supercolonies' that extend for hundreds, or even thousands, of kilometres (Newell & Barber 1913; Way *et al.* 1997; Tsutsui *et al.* 2000; Tsutsui & Case 2001; Giraud *et al.* 2002). Intraspecific aggression and territoriality are absent among nests within these supercolonies and individuals can move freely among different nest sites within the same supercolony. In contrast, native populations of Argentine ants display higher levels of aggression and territorial behaviour over smaller distances (tens to hundreds of metres; Tsutsui *et al.* 2000; Tsutsui & Case 2001). Because individuals that cross these colony boundaries are attacked by ants from foreign colonies, dispersal (particularly by reproductive females) may be spatially restricted.

Pre-existing colonies give rise to new colonies via 'colony budding', when one or more queens leaves the natal colony with a group of workers, disperses a short distance on foot and establishes a new nest nearby. Because newly produced Argentine ant queens do not disperse via nuptial flights, and are instead adopted into their natal colony (Newell & Barber 1913; Markin 1970a), the spread of *Wolbachia* infection through populations may be limited by the reduced dispersal of host females.

Here we examine the spatial and temporal distribution of *Wolbachia* infection in both the native and introduced ranges of the Argentine ant using data from two *Wolbachia* genes. We consider the roles that geographical and behavioural boundaries in the host population play in the distribution of this bacterial infection. We also use phylogenetic analysis to clarify the evolutionary history of *Wolbachia* and to characterize how different strains are distributed within and among populations of Argentine ants. Finally, we examine patterns of transmission within and among species by comparing the *Wolbachia* that infects Argentine ants to that in other insects.

## Materials and methods

### Sampling sites

We collected Argentine ants from 1997 to 2002 at 11 sites in their native range (Argentina) and from locations throughout their introduced range (Fig. 1; Table 1). Workers were typically collected from foraging trails or from shallow

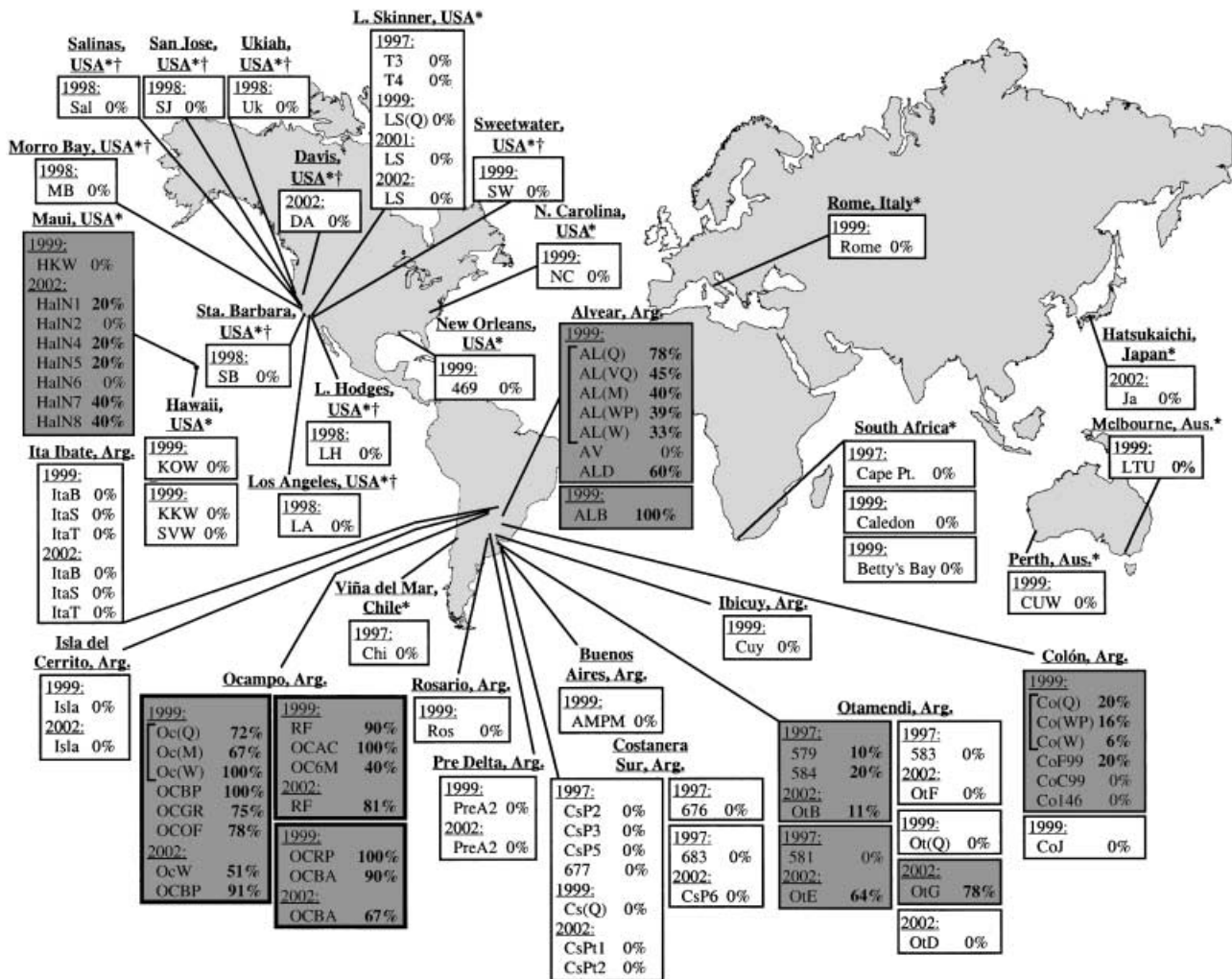


Fig. 1 The spatial and temporal distribution of *Wolbachia* infection in native and introduced populations of the Argentine ant (*Linepithema humile*). Each colony is represented by a box; individual nests, year and infection frequency are shown inside each box. Infected colonies are noted by shading whereas uninfected colonies are not shaded. Colonies infected with supergroup A *Wolbachia* are shown in boxes with a thin border; colonies infected with supergroup B are shown in boxes with a bold border. Asterisks denote introduced populations; daggers denote nests that belong to the large Californian supercolony. When multiple castes were examined in the nest, the caste is indicated in parentheses (Q = dealate queen, VQ = virgin [alate] queen, M = male, W = worker); caste is not labelled for samples in which only workers were examined.

excavations of nest sites. At five sites in the native range and one site in the introduced range we collected colony series that included queens, males, workers and brood by excavating large colony fragments and flooding the ants slowly from the soil.

Because Argentine ant colonies are networks of numerous interconnected nests (polydomous), we identified the location of colony boundaries by using a standard behavioural assay (Suarez *et al.* 1999; Holway *et al.* 1998). We paired individual workers in a neutral arena for 5 min and scored interactions on a scale from 1 to 4: 1 = touch (physical contact, but no aggressive response; includes antennation and trophallaxis), 2 = avoid (after touching, one or both of the ants recoils and retreats), 3 = aggression (attack

by one or both of the workers; includes lunges, biting and pulling of legs and antennae), and 4 = fighting (includes prolonged biting and pulling and the use of chemical defenses). Two nests were considered members of different colonies if at least one behavioural assay escalated to a score of 4, or if the mean aggression score across trials was greater than 1.6. This behavioural assay has a high repeatability and a low variance among trials within the same nest pairing (Tsutsui *et al.* 2000). We used behavioural assays to determine the colony membership of all nests sampled in California, Hawai'i, Maui and Argentina. We did not conduct behavioural assays among the South African sites; however, previous genetic analysis has shown that two of the sites (SA1 and SA2) are genetically similar

**Table 1** Sampling locations, location abbreviations, dates, castes and sample sizes in the native and introduced ranges

Native range						Introduced range					
Site	Colony	Nest	Year	Caste	<i>n</i>	Site	Colony	Nest	Year	Caste	<i>n</i>
Argentina						California, USA					
Alvear	AL1	AL	1999	queen	18	Ukiah	SC	UK	1998	worker	10
	AL1	AL	1999	virgin queen	20	Davis	SC	DA	2002	worker	11
	AL1	AL	1999	male	10	San Jose	SC	SJ	1998	worker	10
	AL1	AL	1999	worker pupae	17	Salinas	SC	SAL	1998	worker	10
	AL1	AL	1999	worker	18	Morro Bay	SC	MB	1998	worker	10
	AL1	AV	1999	worker	10	Santa Barbara	SC	SB	1998	worker	10
	AL1	ALD	1999	worker	10	Los Angeles	SC	LA	1998	worker	10
	AL2	ALB	1999	worker	10	Sweetwater Res.	SW	SW	1999	worker	10
Colón	CO1	CO	1999	queen	10	Lake Hodges	LH1	LH	1998	worker	10
	CO1	CO	1999	worker	18	Lake Skinner	LS1	T3	1997	worker	15
	CO1	CO	1999	worker pupae	19		LS1	T4	1997	worker	15
	CO1	COC99	1999	worker	10		LS1	LS	1999	queen	9
	CO1	COF99	1999	worker	10		LS1	LS	2001	worker	20
	CO1	CO146	1999	worker	10						
	CO2	COJ99	1999	worker	10						
Ita Ibate	IT1	ItaB	1999	worker	10	Hawai'i, USA					
	IT1	ItaB	2002	worker	10	Kilauea, Hawai'i	HI1	KOW	1999	worker	10
	IT1	ItaS	1999	worker	10		HI2	KKW	1999	worker	10
	IT1	ItaS	1999	worker	10		HI2	SVW	1999	worker	10
	IT1	ItaS	2002	worker	10	Haleakala, Maui	HI3	HKW	1999	worker	10
	IT1	ItaT	1999	worker	10		HI4	Hal1	2002	worker	5
Isla del Cerrito	IT1	ItaT	2002	worker	10		HI4	Hal2	2002	worker	5
	IC1	Isla	1999	worker	10		HI4	Hal4	2002	worker	5
	IC1	Isla	2002	worker	10		HI4	Hal5	2002	worker	5
							HI4	Hal6	2002	worker	5
Ocampo	OC1	OCAC	1999	worker	10		HI4	Hal7	2002	worker	5
	OC1	RF	1999	worker	10		HI4	Hal8	2002	worker	5
	OC1	RF02	2002	worker	41						
	OC1	OC6M	1999	worker	10						
	OC2	OCOF	1999	worker	10	Louisiana, USA					
	OC2	OCGR	1999	worker	8	New Orleans	NO1	469	1999	worker	5
	OC2	OC	1999	queen	18						
	OC2	OC	1999	male	18						
	OC2	OC	1999	worker	17	N. Carolina, USA					
	OC2	OC	2002	worker	35	Winston-Salem	NC1	NC	1999	worker	10
Australia	OC2	OCBP99	1999	worker	10						
	OC2	BP	2002	worker	35	Perth	AUS1	CUW	1999	worker	10
	OC3	OCRP	1999	worker	10	Melbourne	AUS2	LTU	1999	worker	5
	OC3	OCBA99	1999	worker	9						
	OC3	BA	2002	worker	36	Chile					

**Table 1** *Continued*

Native range						Introduced range					
Site	Colony	Nest	Year	Caste	<i>n</i>	Site	Colony	Nest	Year	Caste	<i>n</i>
P. N. Pre-Delta	PD1	PreA2	1999	worker	10	Viña del Mar	CHI1	CHI	1997	worker	14
	PD1	PreA2	2002	worker	10						
Rosario	RO1	ROS	1999	worker	10	South Africa					
Ibicuy	IB1	CUY	1999	worker	10	Betty's Bay	SA1	SA1	1999	worker	10
P. N. Otamendi	OT1	579	1997	worker	10	Caledon	SA2	SA2	1999	worker	10
	OT1	584	1997	worker	10	Cape Point	SA3	SA3	1997	worker	10
	OT1	OtB	2002	worker	18						
	OT2	581	1997	worker	9	Italy					
	OT2	OtE	2002	worker	11	Rome	RO	RIW	1999	worker	10
	OT3	583	1997	worker	10						
	OT3	OtF	2002	worker	16	Japan					
	OT4	Ot	1999	queen	10	Hatsukaichi	JA	JA	2002	worker	8
	OT5	OtG	2002	worker	18						
	OT6	OTD	2002	worker	10						
R. E. Costanera Sur	CS1	P2	1997	worker	10						
	CS1	P3	1997	worker	10						
	CS1	P5	1997	worker	10						
	CS1	677	1997	worker	7						
	CS1	CS	1999	queen	18						
	CS1	P1	2002	worker	11						
	CS1	P2	2002	worker	10						
	CS2	676	1997	worker	8						
	CS3	683	1997	worker	8						
	CS3	CSP6	2002	worker	37						
Buenos Aires	BA1	AMPM	1997	worker	10						
			Total	858							
										Total	317

and probably belong to the same colony, whereas the third site (SA3) belongs to a genetically different group (Tsutsui *et al.* 2001).

In the native range, we screened 858 individuals from 55 nest sites (that belonged to 22 behaviourally defined colonies) at 11 sites throughout northern Argentina (Fig. 1; Table 1). We sampled multiple nests at eight sites and multiple colonies at five sites. We sampled the same nests and colonies in multiple years at six sites.

In the introduced range, we screened 317 individuals from 22 locations (33 nests) for *Wolbachia* infection (Fig. 1, asterisks; Table 1). In California, Argentine ants at six of the eight locations belong to the large supercolony that dominates most of the state (Tsutsui *et al.* 2000; Tsutsui & Case 2001; labelled 'SC' in Table 1, noted with daggers in Fig. 1).

### Screening for *Wolbachia* infection

We screened 1175 Argentine ants for the presence of *Wolbachia* using three different genetic markers. We extracted and purified DNA from whole Argentine ants using either (1) a QIAamp tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol or (2) a salting-out procedure (Miller *et al.* 1988; Sunnucks & Hales 1996). We screened these individuals for the presence of *Wolbachia* by using polymerase chain reaction (PCR) to amplify portions of two genes that are used commonly as markers of *Wolbachia* infection: the *Wolbachia* surface protein gene (*wsp*) and a *Wolbachia* cell-cycle gene (*ftsZ*). We amplified a ~600 base pair (bp) section of *wsp* using the primers Wsp81F and Wsp691R (Zhou *et al.* 1998) in a 10- $\mu$ L PCR reaction. We also performed positive controls by including primers specific for a 405 bp segment of the *L. humile* mitochondrial cytochrome B gene (Tsutsui *et al.* 2001) or by amplifying a portion of the *L. humile* 28S gene in parallel with the PCR for *Wolbachia* genes. All individuals were subjected to a secondary screen using the primers *ftsZ*93F and *ftsZ*93R for the *ftsZ* gene (Holden *et al.* 1993). All ants that were negative for *Wolbachia* infection were subjected to a third round of screening, with a second primer set for the *wsp* gene (primer sequences in Jeyaprakash & Hoy 2000). PCR products were electrophoresed on 1.5% agarose gels and bands were visualized with GelStar (Cambrex, East Rutherford, NJ, USA).

Standard PCR methods may fail to amplify target *Wolbachia* sequences in some individuals (Jeyaprakash &

Hoy 2000). To determine the frequency of such false negatives in our data set, we re-screened 163 individuals using a 'long PCR' protocol similar to that of Jeyaprakash & Hoy (2000). We performed PCR reactions using the above primers, but also included in each reaction 1 U of Tgo DNA polymerase (Roche, Indianapolis, IN, USA) and 1 U of *Taq* DNA polymerase. Tgo (which replaces Pwo DNA polymerase) is a 5'-3' DNA polymerase with a 3'-5' exonuclease (proofreading) activity. We used the manufacturer's 5 $\times$  Tgo buffer, reduced the annealing temperature by 4 °C and increased the length of each step in the 30 cycles to 1 min. This method did not reveal any positives that were not also detected using conventional methods (above).

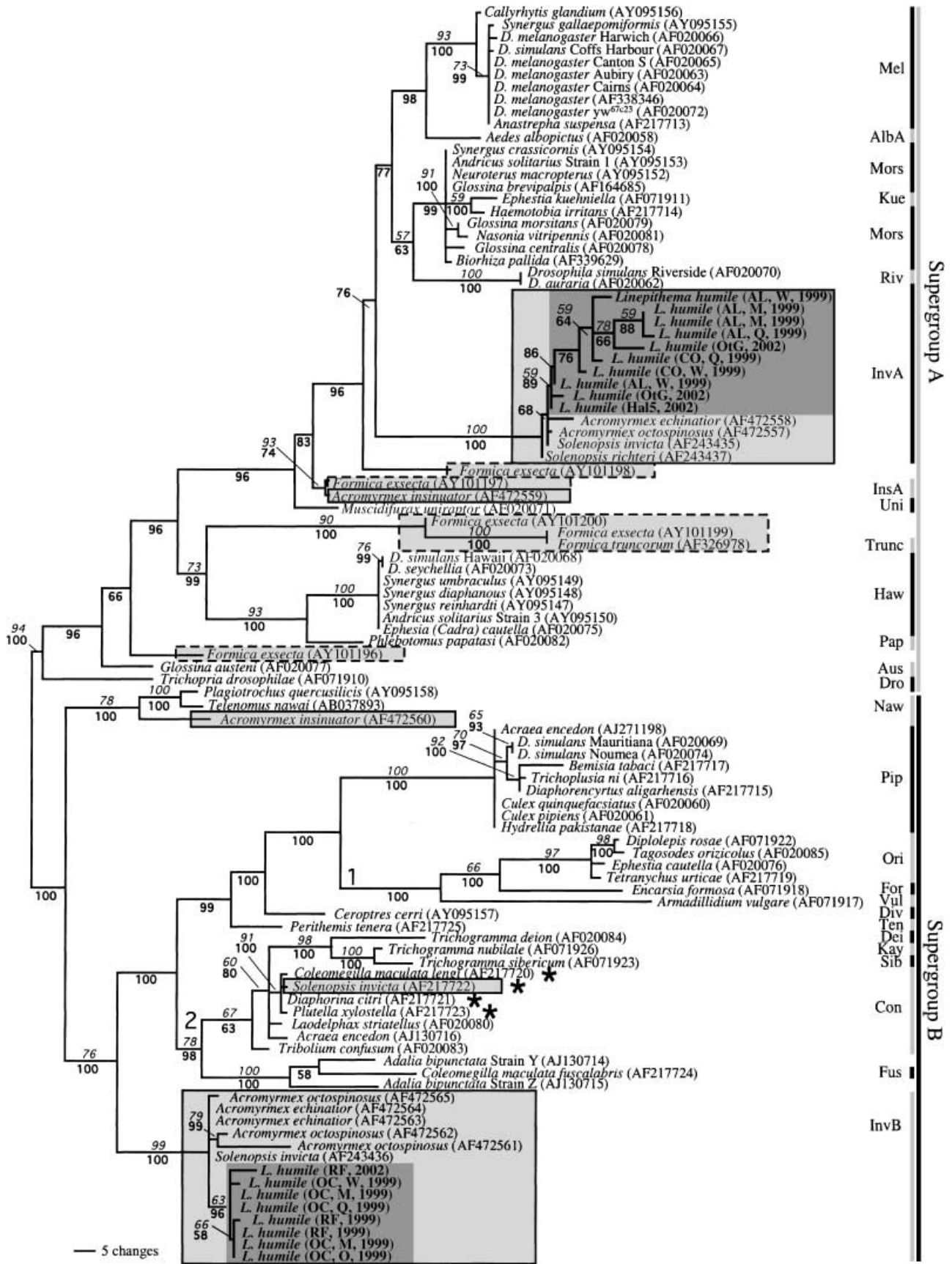
### Phylogenetic analysis

Amplicons from the *wsp* gene were obtained for sequencing by performing PCR reactions as described above, but omitting the positive control and increasing the reaction volume to 50  $\mu$ L. We purified and concentrated PCR products using Millipore Ultrafree-MC Centrifugal filter units (Millipore, Bedford, MA, USA) and sequenced them in both the forward and reverse directions. We aligned these sequences with 83 *wsp* sequences from GenBank (Fig. 2) using the program CLUSTAL W and correcting by eye.

We excluded regions that were hypervariable or that included large gaps (insertions/deletions), due to uncertainty regarding the substitution process. We coded small gaps as missing data. Eight of the sequences (AF020068, AY095149, AY095148, AY095147, AY095150, AF020082, AF020073, AF020075) contained a 50-bp region that appeared to be an indel (sites 61–111). These eight taxa also possessed virtually identical sequences in the second (hypervariable) excluded region (sites 208–244). Finally, a third region of the alignment (sites 375–399) was also hypervariable, unalignable and excluded. To assess the impact of excluding these regions on the topology of the trees, we also performed the analyses with them included; the differences between the resulting trees were minor, and the patterns discussed below were unaffected.

To identify the appropriate substitution rate parameters we first analysed the data set using the program MODELTEST (Posada & Crandall 1998). We then performed phylogenetic reconstructions using maximum likelihood (ML) in the program PAUP\* 4.0b10 (Swofford 1998) using the best-fit model from MODELTEST (HKY + G). We used a

**Fig. 2** Phylogenetic reconstruction of *Wolbachia* using the *wsp* gene. Each *Wolbachia* sequence is labelled with the name of its host species (GenBank Accession no. is indicated in parentheses). The two sets of vertical bars indicate the group (left bars) and strain (right bars) for each *Wolbachia* sequence. Bootstrap support for the maximum parsimony tree (1000 replicates) is shown above each branch in italics and Bayesian posterior probabilities for the maximum likelihood tree are shown below each branch in bold. The *Wolbachia* from Argentine ants are enclosed within dark grey boxes. *Wolbachia* from other ant species are shown in light grey boxes. Boxes with an unbroken border contain New World ant species whereas boxes with a dashed border contain Old World ant species. Asterisks denote a monophyletic clade of *Wolbachia* infecting four sympatric, but distantly related, introduced species in Florida, USA.



Bayesian analysis as implemented in the program MRBAYES version 3 (Huelsenbeck & Ronquist 2001) to calculate posterior probabilities. We ran four Markov chains (one cold, three heated; Huelsenbeck & Ronquist 2001) for 106 generations, sampled every 100 generations, and discarded the first 10 000 generations, after the likelihood values had stabilized. We also performed a phylogenetic reconstruction using maximum parsimony (MP) in PAUP\* (Swofford 1998), and estimated support of the MP tree by conducting 1000 bootstraps.

## Results

### *The spatial and temporal distribution of Wolbachia infection*

Overall, 7 of 317 individuals (2%) in the introduced range were positive for *Wolbachia*. *Wolbachia* was found only in five nests (of 33 examined) in the Argentine ant's introduced range (Fig. 1), and all these nests were in the same location: Haleakala, Maui, USA. Five of the eight nests examined at Haleakala contained at least one infected individual, but the overall infection frequency at this site was low (0% in 1999,  $n = 10$  individuals; 20% in 2002,  $n = 35$  individuals). Interestingly, the primers Wsp81F/Wsp691R and ftsZ93F/ftsZ93R did not detect this strain of *Wolbachia* [even when using the 'long PCR' method of Jeyaprakash & Hoy (2000)], but positives were unequivocal when we used the *wsp* primers of Jeyaprakash & Hoy (2000).

In the native range, 34% of individuals screened (295 of 858) were positive for *Wolbachia* infection. About 42% (23 of 55) of nests and 41% (nine of 22) of colonies contained infected individuals (Fig. 1). Overall, *Wolbachia* was present in Argentine ants at four of the 11 sites in the native range.

*Wolbachia* was present at both sites on the Rio Uruguay (Colón and Alvear; Fig. 1), but not all nests were infected. At Alvear, both of the sampled colonies contained infected individuals, and one of the colonies contained both infected ( $n = 2$ ) and uninfected ( $n = 1$ ) nests. The distance between the uninfected nest and the closest infected nest (113 m) was not substantially different than the shortest distance between the two infected nests (96 m). At the other site on the Rio Uruguay (Colón; Fig. 1), one of the two colonies contained infected individuals and, as at Alvear, the infected colony contained both infected and uninfected nests. Remarkably, one of the uninfected nests (CO1) was < 10 m away from an infected nest in the same colony (CO(Q) and CO(W)).

The overall frequency of infection appeared to be lower in Argentine ants from the Rio Paraná. Two sites contained infected ants (Ocampo and Otamendi; Fig. 1), and ants at three other sites were uninfected (Isla del Cerrito, Rosario and Pre-delta). At Ocampo, all nests in all the colonies were infected in both years that we sampled this site (1999 and

2002). The spatial and temporal distribution of *Wolbachia* infection was more variable at Otamendi: three colonies were infected and three were not (Fig. 1). Of the three infected colonies that we examined in multiple years, one was infected in both 1997 and 2002, one was uninfected in 1997 but was infected in 2002 and the third was uninfected in both 1997 and 2002.

None of the individuals from the nests in the Buenos Aires area (Buenos Aires and Costanera Sur; 95 individuals; nine nests) were infected with *Wolbachia* in any of the years (1997, 1999 and 2002).

### *Patterns of infection within nests*

Because a previous study (Wenseleers *et al.* 2002) showed that *Wolbachia* infection may be cleared from the worker caste, it is important to note that infection frequencies within the worker caste may not reflect the frequency of infection in other castes.

However, when we screened ants from the nest series we found that, in infected nests, some individuals from all castes were infected with *Wolbachia* (Fig. 1). Although there appeared to be some degree of variation in infection frequency among castes, the small sample sizes within colonies precluded rigorous statistical analysis of the hypothesis that infection frequency varies across caste.

### *Phylogenetic patterns*

Many previous studies have shown that there are two major supergroups (occasionally referred to as 'strains') of *Wolbachia*, supergroup A and supergroup B. Within each of these supergroups, numerous groups (or 'strains') have been defined, based on the degree of sequence similarity among members of a particular clade (Stouthamer *et al.* 1999). Our analysis confirms the monophyly of all of these groups except one (Mors; Fig. 2).

Overall, the topologies of the MP and ML trees were highly congruent. The primary difference involved the placement of two clades: one formed by groups Ori, For and Vul and a second clade that included groups Dei, Kay, Sib and Con (labelled 1 and 2, respectively, in Fig. 2). In the ML phylogeny, the Pip group and the Ori/For/Vul clade were sister groups, and the Dei/Kay/Sib/Con clade was basal to these two. In the MP phylogeny (not shown) the Pip group and the Dei/Kay/Sib/Con clade were sister groups, and the Ori/For/Vul clade was basal to them.

Several interesting patterns emerged from the phylogenetic analysis of *Wolbachia* in Argentine ants. First, all *Wolbachia* from *L. humile* fell into one of two monophyletic groups: one group of supergroup A *Wolbachia* and one of supergroup B *Wolbachia* (Fig. 2, dark shading). Second, the two supergroups of *Wolbachia* never co-occurred at the same site, and the populations infected by the different

supergroups were separated by > 250 km. In the native range, all Argentine ants infected with supergroup A were from either (1) the eastern side of the geographical range, at sites on the Rio Uruguay (Alvear and Colón) or (2) the southern end of the Rio Paraná (Otamendi). The single infected population in the introduced range (Haleakala, Maui) was also infected with supergroup A *Wolbachia*. In contrast, all Argentine ants infected with supergroup B were collected from Ocampo, a site that is > 600 km upstream of Otamendi on the Rio Paraná (Fig. 1). Although there appeared to be some variation among *Wolbachia* from the same colony (e.g. colonies at Alvear and Otamendi; Fig. 2), the low bootstrap values and posterior probabilities provide little support for the hypothesis that these are actually different, distinct lineages of the bacterium. Rather, a combination of small differences in sequence and missing data at the 5' or 3' ends of may have produced these apparent differences.

Based on the *wsp* sequences, the *Wolbachia* in Argentine ants appeared to be extremely similar to *Wolbachia* in other New World ants (Fig. 2; light grey boxes with unbroken border). Specifically, the supergroup A *Wolbachia* from Argentine ants formed a well-supported monophyletic group with other supergroup A *Wolbachia* from native populations of *Solenopsis invicta* (the red imported fire ant), *S. richteri* (the black imported fire ant), *Acromyrmex octospinosus* (a leafcutter ant) and *A. insinuator* (a social parasite) (Fig. 2). Similarly, the supergroup B *Wolbachia* from Argentine ants formed a well-supported monophyletic group with *Wolbachia* from native populations of *S. invicta*, *A. octospinosus*, *A. insinuator* and *A. echinator* (a leafcutter ant) (Fig. 2). All these ant species were collected in either Argentina (*Solenopsis* spp.; Shoemaker *et al.* 2000) or Panama (*Acromyrmex* spp.; Van Borm *et al.* 2003).

Only three of the 31 *Wolbachia* sequences from New World ants did not fall into one of these two groups (Fig. 2). Two of the exceptions were *Wolbachia* from *A. insinuator*, one in supergroup A (group *InsA*) and one in supergroup B (group *Naw*). The third exception was *Wolbachia* found in *S. invicta* from a population in its introduced range (Florida, USA; Jeyapakash & Hoy 2000).

In contrast to the similarity among *Wolbachia* from New World ants, *Wolbachia* from Old World ants (*Formica exsecta* and *F. truncorum*) were dispersed throughout the supergroup A clade (Fig. 2; light grey boxes with dashed border). *Wolbachia* from at least four different groups infect ants in the genus *Formica*. One of these, from *Wolbachia* group *InsA*, also infected a New World ant, *A. insinuator*.

## Discussion

Although *Wolbachia* has been found in over 90 ant species (e.g. Wenseleers *et al.* 1998; Jeyapakash & Hoy 2000; Shoemaker *et al.* 2000; Van Borm *et al.* 2001; Wenseleers

*et al.* 2002; Kittayapong *et al.* 2003; Reuter and Keller 2003; Van Borm *et al.* 2003), virtually nothing is known about the interactions between *Wolbachia* and its ant hosts, the causes and consequences of infection, the modes of bacterial transmission within and among ant species or even the identity of the *Wolbachia* group(s) that infects most of these species (but see Wenseleers *et al.* 2002). The results of our study show how the history of invasion, population structure and social organization of the host species can influence the distribution of *Wolbachia* infection. This work also provides evidence for host specialization by two lineages of *Wolbachia*, and shows that frequent, recent horizontal transmission of the *Wolbachia* infection has occurred in at least one other lineage. Finally, the pattern of *Wolbachia* infection in the native vs. introduced ranges of Argentine ant hosts suggests that the near absence of infection may have contributed to the success of invasive populations.

### Spatial patterns of infection — native vs. introduced range

In the native range, the pattern of infection among neighbouring nests is consistent with low levels of queen dispersal within and among colonies. At three sites (Otamendi, Colón and Alvear), *Wolbachia*-infected colonies were adjacent to uninfected colonies, a pattern that persisted at Otamendi, the only site of these three that we sampled over multiple years. If *Wolbachia*-infected queens dispersed frequently across colony boundaries and joined foreign colonies then infection would spread locally as uninfected colonies received *Wolbachia* from their infected neighbours. The reproductive biology of Argentine ant queens also suggests that they probably do not infect distant nests with *Wolbachia*: although Argentine ant queens possess wings before mating they rarely, if ever, disperse by flying (Newell & Barber 1913; Markin 1970a). Because *Wolbachia* is transmitted maternally, male gene flow could occur among colonies without spreading the bacterium. Similarly, because Argentine ant workers are completely sterile (Markin 1970b), the accidental exchange of workers or worker brood among colonies would probably not spread infection across colony boundaries. Even within colonies, movement of queens and workers among different nest sites appears to be limited, as shown by the presence of both infected and uninfected nests within the same colony at some sites (Alvear, Colón, and Otamendi).

The absence of *Wolbachia* in virtually all introduced populations matches the results of a previous study of native and introduced *S. invicta* and *S. richteri* populations (Shoemaker *et al.* 2000). *Solenopsis* from several sites in the native range (Argentina and Brazil) carried *Wolbachia*, whereas ants from the introduced range (Georgia, USA and Mississippi, USA) lacked it (Shoemaker *et al.* 2000; although another study reported *Wolbachia* in introduced *S. invicta*; see below).

The discovery of *Wolbachia* in Argentine ants at Haleakala (Maui) is somewhat surprising in light of the evidence that this population arose after a series of intermediate introductions to other parts of the introduced range (Tsutsui *et al.* 2001). The similarity of this *Wolbachia* to the supergroup A *Wolbachia* in South America (Fig. 2) suggests that the Argentine ants at Haleakala descended from historically infected populations, and that this infection is not the result of recent horizontal transfer of *Wolbachia* from other host taxa. However, the infection at Haleakala could be either the product of additional introductions of Argentine ants directly from the native range (either before or after 1999) or from another introduced population that has not yet been screened for *Wolbachia*. It is also important to note that the collection sites at Haleakala were not exactly the same in 1999 and 2002, so the difference in infection frequency between these samples may reflect spatial variation rather than temporal variation in the abundance of *Wolbachia*.

#### Phylogenetic patterns of *Wolbachia* infection

Previous studies have shown that *Wolbachia* from ants cluster together in several distinct lineages (Van Borm *et al.* 2003). The evidence we present here confirms and extends this hypothesis. Although *Wolbachia* from ants in the Old World (*F. truncorum* and *F. exsecta*) are distributed patchily across supergroup A (Fig. 2), almost all the *Wolbachia* found in New World ants belong to one of two groups, *InvA* and *InvB* (Fig. 2). This pattern suggests that these two lineages of *Wolbachia* may have become specialized parasites of New World ants. Additional studies that examine other Old World ants will be necessary to determine if a similar pattern exists there as well.

Our phylogenetic analysis also shows that closely related non-ant hosts do not appear to be infected by closely related lineages of *Wolbachia*, suggesting that *Wolbachia* in non-ant arthropods typically do not specialize on particular taxonomic groups (although previous studies have shown that there does appear to be some degree of host specialization in *Wolbachia* that infect isopod crustaceans; Bouchon *et al.* 1998). It is important to note, however, that many of the known *Wolbachia* hosts are laboratory strains of insects. Therefore, if horizontal transmission among hosts frequently occurs in captivity, the phylogeny may not reflect the true distribution or identity of *Wolbachia* in natural populations of these species.

#### Horizontal transmission

Although a previous study discovered *Wolbachia* in introduced *S. invicta* in North America (Jeyaprakash & Hoy 2000; Fig. 2), several lines of evidence suggest that this infection is probably the result of recent horizontal

transmission from another arthropod host rather than continued infection from historically infected *S. invicta* populations. First, the three most similar *Wolbachia* sequences are from extremely distantly related host species that occur in sympatry with *S. invicta* in Florida (marked with asterisks in Fig. 2): *Diaphornia citri* (the Asian citrus psyllid), *Plutella xylostella* (the diamondback moth) and *Coleomegilla maculata lengi* (the 12-spotted ladybird beetle). Moreover, like *S. invicta*, these species are not native to Florida, but instead are introduced, each from a different continent (*S. invicta*, South America, Callcott & Collins 1996; *D. citri*, Asia & Mead 1977; *P. xylostella*, Europe, Metcalf & Metcalf 1993; *C. maculata*, northern North America, Hoffmann & Frodsham 1993). Thus, the occurrence of multiple, recent horizontal transfers of *Wolbachia* is the most parsimonious explanation for both the presence of *Wolbachia* in this population of *S. invicta* and for the similarity of the *Wolbachia* in these different sympatric, introduced species. The plausibility of this scenario is strengthened by reports that horizontal transmission can occur via multiple routes in other taxa, such as among *Trichogramma* wasps via common food sources (Huigens *et al.* 2000) and between parasitoids and their hosts (Vavre *et al.* 1999).

#### *Wolbachia* and the enemy-release hypothesis

The enemy-release hypothesis proposes that the success of many invasive species stems from the absence of coevolved predators, competitors and parasites in the introduced range. Recent studies provide support for the enemy-release hypothesis in a variety of taxa, and identify numerous pathogens and parasites that may limit the success of invasive species in their native range (Mitchell & Power 2003; Torchin *et al.* 2003). The absence of *Wolbachia* in most introduced populations of the three invasive ants that have been studied (*L. humile*, *S. invicta* and *S. richteri*) is consistent with predictions of the enemy-release hypothesis. Although the potential fitness impacts of *Wolbachia* infection have not yet been examined in these invasive ants, studies of the noninvasive ant *F. truncorum* suggest that *Wolbachia* infection may impose a metabolic burden, resulting in the production of fewer sexuals in infected colonies (Wenseleers *et al.* 2002). Although other mechanisms, such as the origin of unicoloniality in introduced populations of the Argentine ant, contribute to the success of invasive ants (Holway *et al.* 1998; Tsutsui *et al.* 2000), such processes may work in concert with the escape from natural enemies to promote population growth in the introduced range. Studies that quantify the impact of *Wolbachia* infection on invasive ants will be necessary to determine the role that the absence of this bacterium may have played in their success, and could inform programs seeking to use *Wolbachia* as a potential biological control agent.

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