



## Recent range expansion of the Argentine ant in Japan

Maki N. Inoue<sup>1\*</sup>, Eiriki Sunamura<sup>2</sup>, Elissa L. Suhr<sup>3</sup>, Fuminori Ito<sup>4</sup>, Sadahiro Tatsuki<sup>2</sup> and Koichi Goka<sup>1</sup>

<sup>1</sup>National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, 305-8506, Japan, <sup>2</sup>Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, 1-1-1 Bunkyo-ku, Tokyo, 113-8657, Japan, <sup>3</sup>Australian Centre for Biodiversity, School of Biological Sciences, Monash University, Clayton, Vic, 3800, Australia, <sup>4</sup>Laboratory of Entomology, Faculty of Agriculture, Kagawa University, Ikenobe, Miki, 761-0795, Japan

### ABSTRACT

**Aim** The Argentine ant, *Linepithema humile*, has been spreading via human activities from its native range in South America across much of the globe for more than a century. This invasive ant was first detected in Japan in 1993. Its successful world-wide expansion is attributed to a social structure, namely supercoloniality, whereby individuals from separate nests cooperate. Here, we examined the genetic structure of *L. humile* populations to understand its invasion history.

**Location** Japan.

**Methods** We analysed mitochondrial DNA of *Linepithema humile* workers from native and other introduced populations and then integrated previously registered sequences.

**Results** Sequencing revealed six haplotypes distributed across its introduced ranges, of which five were present in Japan. The first haplotype was shared by the dominant Japanese, European, North American, Australian and New Zealand supercolonies; the second by the *Kobe C* supercolony and a Florida population; and the third by the *Kobe B* and secondary Californian supercolonies and North Carolina colonies. The remaining three haplotypes were each restricted to the *Kobe A*, *Tokyo* and *Catalonian* supercolonies, respectively. Each of the five mutually antagonistic supercolonies was fixed for one of the five haplotypes, and multiple supercolonies were found within a small area.

**Main conclusions** The large number of haplotypes found in Japan likely reflects the strong propagule pressure of *L. humile* resulting from the fact that the country is one of the top five importers of trade commodities world-wide. The short invasion history of *L. humile* in Japan could explain the maintenance of genetic diversity of each independent introduction. In addition, our sampling mostly occurred at major international shipping ports that are likely to be primary sites of introduction. The several recently established *L. humile* populations within a small area in Japan provide an opportunity to identify the sources of introduction and the local patterns of spread.

### Keywords

Biological invasions, invasion history, *Linepithema humile*, mitochondrial DNA, social insects, supercolony.

\*Correspondence: Maki N. Inoue, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan. E-mail: inoue.maki@nies.go.jp

### INTRODUCTION

Invasive alien species threaten native biodiversity world-wide (Mack *et al.*, 2000) and cause significant economic losses in agriculture, forestry and other industries (Vitousek *et al.*, 1996). The increasing global exchange of commodities supports the accidental transport of alien species through

commercial trade pathways and will likely lead to higher numbers of alien species in most parts of the world (Hulme, 2009).

The Argentine ant, *Linepithema humile* (Mayr), native to South America, is one of the world's most damaging invasive species. It has invaded every continent but Antarctica, particularly in areas with a Mediterranean climate (Suarez *et al.*,

2001; Roura-Pascual *et al.*, 2011). In the introduced ranges, *L. humile* competitively displaces or disrupts local arthropod communities (Human & Gordon, 1996; Holway, 1999) and imperils other species in the ecosystem, such as native plants that depend on native ants for seed dispersal (Christian, 2001; Rowles & O'Dowd, 2009). The species also causes agricultural damage by protecting plant pests from predators and parasitoid (Ness & Bronstein, 2004; Daane *et al.*, 2007).

Colonies of *L. humile* are highly polygynous (i.e. many reproductive queens) and polydomous (i.e. many nests) and possess a unique social structure, supercoloniality, whereby individuals mix freely among separated nests (Helanterä *et al.*, 2009). In the species' native range, *L. humile* is characterized by mutually antagonistic colonies but can form small supercolonies tens to hundreds of meters in size that are genetically differentiated from one another (Heller, 2004; Pedersen *et al.*, 2006). In contrast, introduced *L. humile* populations in California, Europe, Australia, New Zealand and Japan form large supercolonies that spread across tens to thousands of kilometres (Tsutsui *et al.*, 2000; Giraud *et al.*, 2002; Corin *et al.*, 2007a; Sunamura *et al.*, 2007, 2009a; Suhr *et al.*, 2011). Within these supercolonies, workers are genetically similar (Tsutsui & Case, 2001; Jaquiere *et al.*, 2005) and display no aggression toward nestmates (Holway *et al.*, 1998). The widespread cooperation and formation of massive supercolonies is considered to contribute to the invasion success of *L. humile* (Tsutsui *et al.*, 2000).

In Japan, *L. humile* was first reported in 1993 (Sugiyama, 2000) and is now present in several parts of the country (Okaue *et al.*, 2007). The majority of introduced populations form a single widespread supercolony (*Japanese main*), while a few small mutually aggressive secondary supercolonies (*Kobe A*, *Kobe B*, *Kobe C*, and *Tokyo*) have been detected (Sunamura *et al.*, 2007, 2009a; M. Inoue unpublished). To prevent further range expansion of *L. humile*, early detection, rapid response systems and control measures are required. A fundamental component of such prevention is identifying the pathways of introduction and movement of introduced populations into and across Japan. Although pathway analysis of intentionally introduced species is straightforward in cases of deliberate release, unintentional releases are much less traceable.

Molecular markers are useful for studying the invasion history and population structure of invasive species (e.g. Durka *et al.*, 2005; Grapputo *et al.*, 2005; Cameron *et al.*, 2008). Microsatellite markers have often been used as a tool for investigating population genetics of *L. humile* (e.g. Tsutsui *et al.*, 2000). However, microsatellites exhibit a high mutation rate and are consequently highly polymorphic even within a colony. In addition, introduced *L. humile* populations may experience genetic drift (Tsutsui *et al.*, 2000; Tsutsui & Case, 2001), and there could be high divergence rates between introduced populations and their native source. Therefore, microsatellites are less applicable for tracing this ant's expansion across the world. In contrast, mitochondrial DNA (mtDNA) lacks recombination and is maternally

inherited, making it an ideal tool for investigating the invasion histories of introduced populations that require founding queens (Tsutsui *et al.*, 2001; Corin *et al.*, 2007b).

In this study, we used mtDNA to examine the population structure of *L. humile* populations in Japan and other introduced populations world-wide. We then integrated previously registered *L. humile* sequences from native and other introduced populations (Vogel *et al.*, 2009, 2010) with our genetic data and reanalysed the data set in an attempt to understand the invasion history of *L. humile*.

## METHODS

### Sample collection

We collected *L. humile* workers from 20 populations in Japan and 18 other introduced populations world-wide: 14 from North America, two from Europe, one from Australia and one from New Zealand (Table 1). Specimens were collected from 2005 to 2011 and stored in microtubes at  $-28^{\circ}\text{C}$ . The Japanese samples were collected from five supercolonies (*Japanese main*, *Kobe A*, *Kobe B*, *Kobe C*, and *Tokyo*; Sunamura *et al.*, 2007, 2009a; Hirata *et al.*, 2008; M. Inoue, pers. obs.), and one additional population (JT3). We do not report the supercolony of JT3 because the population could not be found owing to eradication. The European samples came from the *European main* and *Catalonian* supercolonies in Spain (Giraud *et al.*, 2002). The North American samples were collected from four Californian supercolonies (*Californian large*, *Lake Hodges*, *Lake Skinner*, and *Sweetwater*; Tsutsui *et al.*, 2003), and four North Carolina colonies (*RTPb*, *RTPc*, and *FOR*; Vasquez & Silverman, 2008; and Wilmington), two Hawaii colonies (HM1 and HM2; Cole *et al.*, 1992) and one colony from Florida (AF) and Georgia (AG), respectively. The Australian and New Zealand samples came from the *Australian* and *New Zealand* supercolonies, respectively (Corin *et al.*, 2007a; Suhr *et al.*, 2011).

To identify the supercolony to which the populations belong (Table 1), we sampled workers in Tokyo and Tokushima and conducted worker-worker aggression tests. One worker from a population and another from a previously identified supercolony were randomly selected and placed in a plastic dish (4 cm diameter) and observed for 5 min. To quantify their behaviour, we scored each contact using a 0–4 scale modified from Suarez *et al.* (1999) as follows: 0 = ignoring, 1 = avoidance or antennation, 2 = dorsal flexion, 3 = aggression and 4 = fighting. For each population and supercolony combination, six pairs were tested. According to aggression tests, workers from the JT01 population showed a high level of aggression towards all four Japanese supercolonies and we named the new supercolony *Tokyo*. The other three populations were identical to the previously known supercolonies: JTO2 to *Japanese main*; JT1 to *Kobe A*; and JT2 to *Kobe B*. One population in Japan and five in the USA for which aggression tests have not yet been conducted are not identified by a supercolony name in Table 1.

**Table 1** *Linepithema humile* sample information: source country, site location, location code (unique for each population), supercolony name and number of workers per site from which mtDNA sequences were obtained (*n*)

Country	Site	Location code	Supercolony name	<i>n</i>	Haplotype
Japan	Ota, Tokyo	JTO1	Tokyo*	2	LH5
	Ota, Tokyo	JTO2	Japanese main*	2	LH1
	Yokohama, Kanagawa	JY	Japanese main	4	LH1
	Shizuoka, Shizuoka	JSS	Kobe A	2	LH2
	Kagamigahara, Gifu	JG	Kobe B	12	LH3
	Tahara, Aichi	JA	Japanese main	18	LH1
	Kyoto, Kyoto	JKF	Kobe B	2	LH3
	Osaka, Osaka	JO	Japanese main	18	LH1
	Kobe, Hyogo	JKA	Kobe A	6	LH2
	Kobe, Hyogo	JKB	Kobe B	18	LH3
	Kobe, Hyogo	JKC	Kobe C	9	LH4
	Kobe, Hyogo	JKD	Japanese main	9	LH1
	Tokushima, Tokushima	JT1	Kobe A†	2	LH2
	Tokushima, Tokushima	JT2	Kobe B†	2	LH3
	Tokushima, Tokushima	JT3‡		2	LH2
	Hiroshima, Hiroshima	JHHR	Japanese main	12	LH1
	Hatsukaichi, Hiroshima	JHHT	Japanese main	4	LH1
	Otake, Hiroshima	JHO	Japanese main	4	LH1
	Iwakuni, Yamaguchi	JYI	Japanese main	16	LH1
	Yanai, Yamaguchi	JYY	Japanese main	4	LH1
USA	Davis, California	AC	Californian large	2	LH1
	Los Angeles, California	AL	Californian large	2	LH1
	San Diego, California	ASD1	Lake Hodges	2	LH3
	San Diego, California	ASD2	Lake Skinner	2	LH3
	San Diego, California	ASD3	Sweetwater	2	LH3
	San Diego, California	ASD4	Californian large	6	LH1
	Raleigh, North Carolina	ANC1	RTPb	20	LH3
	Raleigh, North Carolina	ANC2	RTPc	2	LH3
	Winston-Salem, North Carolina	ANC3	FOR	2	LH3
	Wilmington, North Carolina	ANC4		2	LH3
	Gainesville, Florida	AF		4	LH4
	Huston, Georgia	AG		4	LH1
	Area 1 (2800–2880 m a.s.l.) <sup>§</sup> , Maui, Hawaii	HM1		8	LH1
	Area 2 (2070–2160 m a.s.l.) <sup>§</sup> , Maui, Hawaii	HM2		8	LH1
Australia	Melbourne, Victoria	AM	Australian	12	LH1
New Zealand	Auckland	NZA	New Zealand	3	LH1
Spain	Cerdanyola, Barcelona	SBC	European main	4	LH1
	Sant Cugat del Valles, Barcelona	SBS	Catalonian	4	LH6

\*The 5-min worker–worker aggression tests of each pair (*n* = 6) were conducted by M. Inoue (pers. obs.).

†The 5-min worker–worker aggression tests of each pair (*n* = 6) were conducted by F. Ito (pers. obs.).

‡The aggression tests were not conducted because we could not find the population owing to eradication.

§Area 1 and Area 2 were partitioned by Cole *et al.* (1992).

## DNA analysis

DNA was extracted from 233 individual *L. humile* workers using the method described by Goka *et al.* (2001). After the application of 60 µL of lysis buffer [1 mg mK<sup>-1</sup> proteinase K, 0.01 M NaCl, 0.1 M EDTA, 0.01 M Tris–HCl (pH 8.0), 0.5% Nonidet P-40], each worker was homogenized with a thermal regime of 50 °C for 60 min then 94 °C for 10 min. The homogenate was then diluted with 270 µL TE buffer [0.001 M EDTA, 0.001 M Tris–HCl (pH 8.0)]. Polymerase chain reactions (PCRs) were used to amplify a 1700-bp partial sequence from the cytochrome *c* oxidase subunits I (COI) and II (COII) genes.

Initially, we attempted to amplify this mitochondrial region using universal primer pairs developed by Simon *et al.* (1994). However, amplifications of some fragments were unreliable, so *Linepithema*-specific primers were designed on the basis of some successfully amplified sequences. The three primer sets used were Lh1751 (5'-CCCTCGAATAAATAATATAAG-3') and Lh2329b (5'-GGCAATTATAGCATAGATTATTCC-3'); Lh2195 (5'-TT-GATTTTTTGGACATCCCGAAG-3') and Lh3014 (5'-TTGAAGGGATTTCATCGTATC-3'); and Lh2797 (5'-GAGAAGCTTTATCATCTAAACG-3') and Lh3389b (5'-GGTAGAATCTATTTTAATTCC-3'). These primer sets amplified three partly overlapping fragments, which together gave the

COI–COII sequence. A 524-bp sequence of the mtDNA cytochrome *b* (Cyt *b*) gene was also amplified by the primer set, L-Lhcb and R-Lhcb (Pedersen *et al.*, 2006).

Each 50- $\mu$ L reaction consisted of 1  $\mu$ L of template DNA, 0.2 mM each dNTP, 2 mM MgCl<sub>2</sub>, 1.25 units of *Taq* DNA polymerase (Amplitaq Gold; Applied Biosystems, Foster City, CA, USA) and 0.4  $\mu$ M each primer (Perkin Elmer Applied Biosystems). PCRs were run with a thermal regime of an initial 10 min at 95 °C; 30 cycles of 30 s at 94 °C, 30 s at 46–47 °C and 2 min at 72 °C; and a final 7 min at 72 °C. PCR products were sequenced directly using a BigDye Terminator Version 3.1 Cycle Sequencing Kit and a BigDye XTerminator Purification Kit (Applied Biosystems) on an ABI 3770 DNA analyzer (Applied Biosystems).

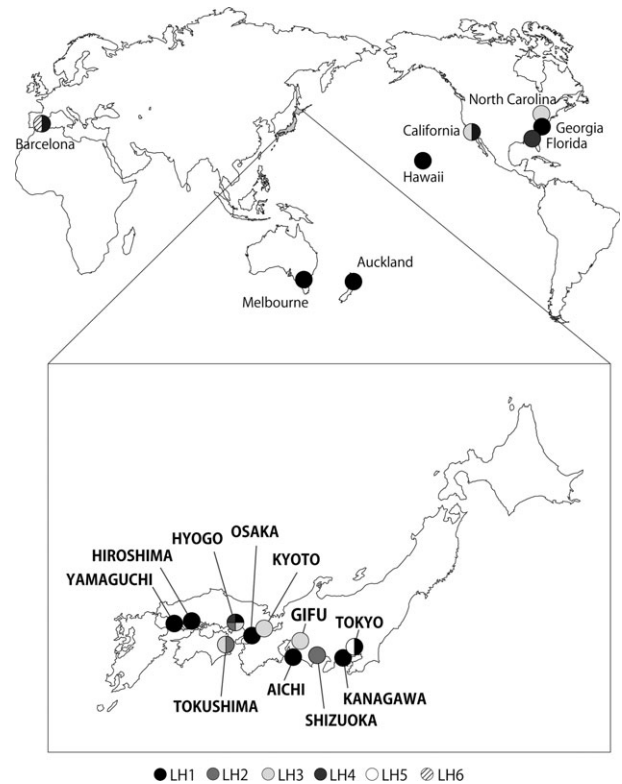
### Data analysis

After manual editing, sequences were aligned using the MEGA 4.0 software package (Tamura *et al.*, 2007) to construct a maximum-parsimony tree for clustering haplotypes. We then collapsed the sequences of all introduced populations to 741 and 524 bp in length to match previously registered COI–COII and Cyt *b* sequences of *L. humile* from native and other introduced populations in GenBank (Vogel *et al.*, 2009, 2010) and analysed phylogenetical relationships among haplotypes. GeneBank accession numbers for H1–H18 are FJ466647–FJ466664 for Cyt *b*, FJ466666–FJ466683 for COI and FJ535653–FJ535670 for COII. Gene accession numbers for *L. oblongum*, used as an outgroup taxon, are FJ496346 for Cyt *b*, FJ496349 for COI and FJ496352 for COII. To test the reliability of each clade on the tree, 1000 bootstrap resamplings were performed.

### RESULTS

The sequences of amplified mtDNA from 233 ants sampled from 38 introduced populations world-wide revealed six haplotypes, five of which were present in Japan (GeneBank accession numbers: AB568481–AB568484 and AB693875 for COI–COII, AB693876–AB693881 for Cyt *b*; Fig. 1). In all analysed individuals, the COI–COII and Cyt *b* gene sequences did not show any deletions or insertions. We found nucleotide substitutions at 47 positions among the six haplotypes. All substitutions were synonymous; 43 substitutions were transitions (11 A→G, 10 G→A, 15 T→C, 7 C→T) and 4 were transversions (A→T, T→A, T→G, C→A).

Haplotype LH1 was shared by populations from the *Japanese main* (JTO2, JY, JA, JO, JKD, JHHR, JHHT, JHO, JYI and JYY), *European main* (SBC), *Californian large* (AC, AL, and ASD4), *Australian* (AM) and *New Zealand* (NZA) supercolonies and populations from Georgia, USA (AG) and Hawaii (HM1 and HM2). Haplotype LH3 was shared by populations from the *Kobe B* (JG, JKF, JKB, and JT2), Californian supercolonies [*Lake Hodges* (ASD1), *Lake Skinner* (ASD2), and *Sweetwater* (SD3)], and North Carolina colonies [*RTPb* (ANC1), *RTPc* (ANC2), *FOR* (ANC3), and



**Figure 1** Geographical distribution of *Linepithema humile* populations sampled. Each colour represents one of the six haplotypes identified in this study.

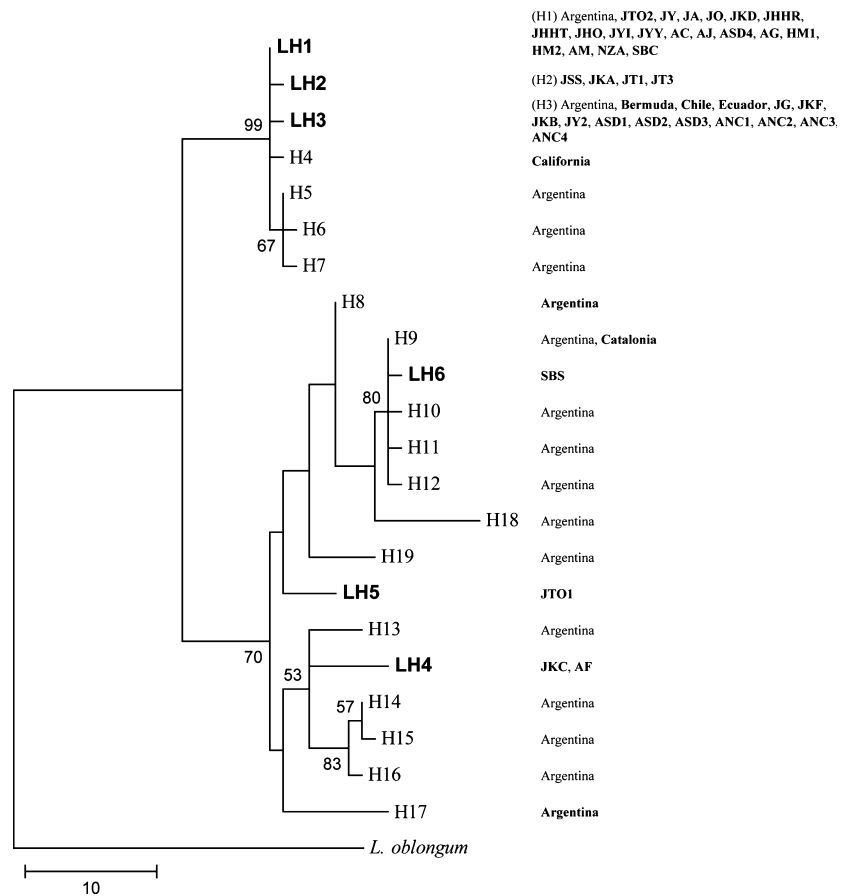
Wilmington (ASD4)]. Haplotype LH2 was found only in populations from the *Kobe A* supercolony (JKA, JSS and JT1) and the Tokushima population (JT3) in Japan, while LH5 was found in the *Tokyo* supercolony (JTO1) from Japan, and LH6 in the *Catalonian* supercolony (SBS) in Spain. Haplotype LH4 was shared by the *Kobe C* (JKC) supercolony and the Florida (AF) population. Each supercolony was fixed for a single haplotype, although in most cases, the sample size per population was very limited.

Two haplotypes, LH1 and LH3 from nearly all introduced populations, were identical to haplotypes previously identified in native populations (Fig. 2). The other four haplotypes, LH2, LH4, LH5 and LH6, were not detected in any native population.

### DISCUSSION

Mitochondrial genetic analyses of *L. humile* revealed the presence of 10 haplotypes in the regions of introduction across the world: Vogel *et al.* (2010) identified seven haplotypes, while we found three new haplotypes (LH4 in Kobe and Florida, LH5 in Tokyo and LH6 in Spain). Each supercolony had a single mitochondrial haplotype except for the *Catalonian* supercolony where all four sampled individuals had another haplotype that differs from the one reported by Vogel *et al.* (2010) by a single base pair. A rare haplotype, H4, has also been found in the *Californian* supercolony in

**Figure 2** Maximum parsimony of the relationships between native and introduced *Linepithema humile* populations by using 741 bp of the mitochondrial COI–COII gene and 524 bp of the cytochrome *b* gene. Bootstrap values exceeding 50% are shown (1000 replicates). Population codes (e.g. JKA) indicate the geographical source and correspond to Table 1. Introduced populations are in bold, and H indicates the haplotype number according to Vogel *et al.* (2010). The outgroup branch length is not to scale.



one individual (Vogel *et al.*, 2010). These second haplotypes (H4 and LH6) may arise from independent introductions of different source populations or mutations that deviate from previously introduced populations.

Our results also showed that the dominant Japanese supercolony has the same haplotype as the dominant European, Californian, Hawaiian, Australian and New Zealand supercolonies. Recently, researchers showed that *L. humile* from these dominant supercolonies were genetically similar in both microsatellite loci and mtDNA (Brandt *et al.*, 2009; van Wilgenburg *et al.*, 2010; Vogel *et al.*, 2010) and had similar hydrocarbon profiles (Brandt *et al.*, 2009). Furthermore, Sunamura *et al.* (2009b) and van Wilgenburg *et al.* (2010) documented an absence of aggression among workers belonging to these dominant supercolonies. Our genetic results also support the idea that *L. humile* forms a vast global supercolony across Europe, North America, Australasia and Japan, with long-distance human-mediated jump-dispersal events distributing the LH1 haplotype world-wide.

Generally, low genetic diversity is observed in introduced populations of invasive species (Grapputo *et al.*, 2005; Ficetola *et al.*, 2008), and the occurrence of bottlenecks and genetic drifts could contribute to genetic differentiation by reducing the number of haplotypes present in a population. For example, reduced genetic diversity has been reported in

the introduced ranges of several invasive alien ant species: *Anoplolepis gracilipes* (Drescher *et al.*, 2007), *Wasmannia auropunctata* (Mikheyev & Mueller, 2007) and *Solenopsis invicta* (Caldera *et al.*, 2008; Ross & Shoemaker, 2008). However, recent studies in invasive species other than ants have found no such reduction, and frequently there is actually an increase in genetic diversity because of multiple introductions (e.g. Wilson *et al.*, 2009). In the case of *L. humile*, genetic diversity is higher in the native populations than in the introduced populations (Suarez *et al.*, 1999; Vogel *et al.*, 2010). Heterogeneous environments in the native range because of intra- and inter-specific competition, pathogen attacks and natural disturbances such as flooding (Vogel *et al.*, 2010) cause population subdivisions of *L. humile*, resulting in a large number of small supercolonies. In the introduced ranges, genetic drift may reduce the genetic diversity of *L. humile* populations. *Linepithema humile* occurs at high abundance in urban areas (Suarez *et al.*, 1998; Holway *et al.*, 2002), thus a few adaptive supercolonies extend their distribution into the homogenous artificial environments.

Across the introduced ranges, *L. humile* populations in Japan have the highest genetic diversity in terms of haplotype number and each of the five mutually antagonistic supercolonies has a different haplotype. In contrast, we found four haplotypes among *L. humile* populations from the USA, and

some behaviourally defined supercolonies were fixed for the same haplotype. Only one haplotype has been found in each of the Australian and New Zealand supercolonies and three across Europe (Corin *et al.*, 2007b; Vogel *et al.*, 2010; this study). Furthermore, several supercolonies were found within a small area in Japan: two supercolonies within the ports of Tokyo and Tokushima (M. Inoue, F. Ito, pers. obs.) and four supercolonies within the port of Kobe.

Japan is one of the top five countries for international trade based on import and export values, and thus there are numerous opportunities for repeated *L. humile* introductions. Assuming that each haplotype represents an independent introduction event, the presence of five haplotypes among introduced populations of *L. humile* in Japan shows the occurrence of multiple introductions. Roura-Pascual *et al.* (2011) suggested that the magnitude of internationally traded commodities among countries was not related to the global distributional patterns of *L. humile*. However, the 2007 trade statistics they used likely do not reflect the world trade structure from the 1800s and early 1900s, when *L. humile* first started to be carried around the world (Inoue & Goka, 2009). On the other hand, the large volume of imports has likely intensified the recent propagule pressure of *L. humile* in Japan. Thus, trade volume could explain the larger number of haplotypes found in Japan as well as the USA relative to other sites of introduction, such as New Zealand and Australia (Corin *et al.*, 2007b).

Another reason for the higher genetic diversity of *L. humile* populations in Japan may be their relatively short invasion history of 20–30 years. *Linepithema humile* was introduced much earlier to the USA, where it was first detected at the end of the 1800s in the south-eastern part of the country (Suarez *et al.*, 2001) but not reported in Japan until the 1990s. The levels of intraspecific aggression and numbers of haplotypes may differ between the two countries because of the difference in the stages of invasion. *Linepithema humile* has been present in the USA for more than 120 years, which may have allowed for selection or drift to change gene frequencies relative to initial introduction events. In contrast, the short invasion history of *L. humile* in Japan means that the genetic diversity of each population likely still reflects that of the source population. Therefore, studying populations of *L. humile* in Japan may allow us to estimate the number of founding queens in such primary introductions more accurately than was possible in previous studies (e.g. *L. humile*: Giraud *et al.*, 2002; *S. invicta*: Ross & Shoemaker, 2008). Furthermore, the dominant *Japanese main* and secondary *Kobe B* supercolonies have been spreading from the ports along the coasts as well as into inland regions. If these two supercolonies are superior competitors and displace the other *L. humile* supercolonies, there may be fewer haplotypes in Japan, as is the case in the other introduced regions. For example, the stronger competitive ability of the *European main* supercolony than that of the *Catalonian* supercolony may explain the dominance of the *European main* supercolony in Europe (Abril & Gomez, 2011).

It must be noted that in Japan, we collected *L. humile* samples from most infested areas, including the ports of Tokyo, Osaka and Kobe, which are three of the five major international shipping ports in the country. These ports are likely to be primary sites of introduction for *L. humile* from the native and other introduced ranges. In the USA, Australia and New Zealand, however, most samples were collected some distance away from ports. It is possible that more haplotypes and supercolonies could be found near ports in these other regions. Further research in introduced ranges may contribute to finding new supercolonies, as was the case in South Africa (Mothapo & Wossler, 2011).

The existence of several recently established *L. humile* populations within a small area in Japan allows us to examine the source of introductions and the local pattern of spread. The *Kobe C* supercolony and the Florida population share the same haplotype (LH4), which was not found elsewhere. In addition, populations from the *Kobe B* supercolony exhibit the same haplotype as the secondary Californian supercolonies and North Carolina colonies. According to 2007 trade statistics for the port of Kobe (Bureau of Ports and Harbors, City of Kobe), the top five countries from which agricultural products were imported to Kobe in tonnage (of 5,722,321 t in total) were the USA (41.5%), China (13.2%), Canada (13.0%), the Philippines (12.6%) and Singapore (4.2%). Because *L. humile* has been present in Florida for close to a century (Deyrup *et al.*, 2000), historical, genetic and trade data suggest that the *Kobe C* and *Kobe B* supercolonies originated from a source population transferred stepwise from Argentina to the USA to Japan. We cannot rule out the possibility of a primary introduction from the native range, though. In contrast, the haplotypes found in the *Kobe A* (LH2) and *Tokyo* (LH5) supercolonies were not found in any other native or introduced populations. Thus, those populations are likely independent primary introductions from the native range. The native range and other regions need to be sampled at a far greater scale to identify the source(s) of these two introduced populations.

Populations from the *Kobe A* and *Kobe B* supercolonies have been detected in other parts of Japan. *Kobe A* populations have been found in the ports of Kobe and Tokushima and in Shizuoka city. The Shizuoka population has been found only in the factory of a private beverage-producing company that is separated from the nearest port of Shimizu by 5 km (H. Mori, T. Kishimoto, M.N. Inoue, K. Goka and F. Ito, unpublished data). This company also exchanges products with a factory close to Kobe, Hyogo Prefecture, suggesting that the Shizuoka population originated from the Kobe population via human-mediated jump dispersal on land. The *Kobe A* and *Kobe B* supercolonies are found within the port of Tokushima, which is a minor port whose main international trade partners are China and South Korea, where *L. humile* is absent. There was a passenger ship route between the Tokushima and Kobe ports from 1971 to 1995, suggesting that the Tokushima populations may have established from a translocations of the *Kobe A* and *Kobe B*

supercolonies in the 1990s. The *Kobe B* populations have also been found in inland regions within Kyoto and Gifu Prefectures, where a park improvement project was conducted recently. The Kyoto population is separated by approximately 75 km from the Osaka international port, the closest area where *L. humile* has been established, whereas the Gifu population is about 45 km away from the Nagoya international port, where *L. humile* has not yet become established. This is the first report of a domestic jump-dispersal pathway of *L. humile* across Japan. Early detection of *L. humile* populations will help us understand the pathways of the introduction and movement of invasive species and consequently to prevent further *L. humile* invasions.

The occurrence of five supercolonies within a small area in Japan, unlike the lower diversity in other regions, suggests that the recent expansion of world trade is a likely cause accelerating the global movement of *L. humile* (Inoue & Goka, 2009). The increasing global exchange of commodities and humans will probably lead to further widespread movement of *L. humile* to many parts of the world where it has not yet become established (Roura-Pascual *et al.*, 2004). Consequently, the development of international quarantine systems is urgently needed for preventing future invasions.

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## BIOSKETCH

**Maki N. Inoue** is a postdoctoral researcher at the National Institute of Environmental Studies. Her research interests are

ecology and evolution of invasive social insects, such as bees and ants, and interaction between flowering plants and insects.

**Eiriki Sunamura** earned PhD degree at the University of Tokyo for the studies on the ecology and control of *L. humile*, and now works at Sumitomo Chemical Co., Ltd. as a pesticide researcher.

**Elissa Suhr** is a PhD student at Monash University and visiting scholar at the University of Illinois. Her research interests include biological invasions, population genetics and evolutionary biology, with a focus on ants.

**Fuminori ITO** is a professor of entomology at Kagawa University. His research interests include biology of tropical ants and ecological impact of invasive ants.

**Sadahiro Tatsuki** is Emeritus Professor of the University of Tokyo. His major research field has been insect pheromones from basic science to practical application. Now, in addition to giving regular lectures at several universities, he is the leader of 'ARGANT', an Argentine ant research team at UT.

**Koichi Goka** is a principal researcher at the National Institute. He has promoted the study projects of risk assessments and managements for invasive alien species. He is also interested in the invasive alien parasites and investigates the interaction between collapse of biodiversity and pandemic of emerging diseases.

Author contributions: M.N.I. conceived the ideas for expanding process of *L. humile*, E.S. and S.T. conceived the idea for the multiple introductions of *L. humile* into Japan, M.N.I., E. S., E.L.S., F. I. and K. G. collected the data, M.N.I. and K.G. analyzed the data, and M.N.I. led the writing with contributions from E.L.S. and K.G. and E.S. and S.T. performed preliminary bioassays.

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