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Four mutually incompatible Argentine ant supercolonies in Japan: inferring invasion history of introduced Argentine ants from their social structure

Eiriki Sunamura[1,*], Satoko Hatsumi[2], Satoshi Karino[2], Koji Nishisue[1],
Mamoru Terayama[1], Osamu Kitade[2] & Sadahiro Tatsuki[1]

[1]Graduate School of Agricultural and Life Sciences, the University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan; [2]College of Science, Ibaraki University, Mito, Ibaraki 310-8512, Japan; [*]Author for correspondence (e-mail: aa087004@mail.ecc.u-tokyo.ac.jp / eirikisunamura@yahoo.com; tel.: +81-3-5841-5093; fax: +81-3-5841-5061)

Abstract In recent years, highly invasive ant species successively invaded warm regions of Asia. In Japan, the Argentine ant, *Linepithema humile*, has become established in several coastal regions. This species forms unusual social organizations called supercolonies consisting of numerous mutually non-aggressive nests. We studied the behavioral relationships, similarity of cuticular hydrocarbon profiles (nestmate recognition cue), and genetic relationships among the introduced Argentine ant populations of Japan. The Japanese populations were divided into four behaviorally, chemically, and genetically distinct supercolonies, which may have derived from independent source populations. The result represents the recent trend of increasing invasions of invasive ants to Asia. The discontinuous distribution of one supercolony throughout most of the Japanese range suggests rapid expansion of the supercolony via human-mediated jump dispersal. Meanwhile, localization of the other three supercolonies in Kobe Port provides the first evidence for multiple invasions of distinct supercolonies into a base for international trade.

Key words: Asia, biological invasion, invasion history, Japan, *Linepithema humile*, supercolony

Introduction

Invasions by alien ants have caused a broad range of ecological and economic damage in many parts of the world (Holway et al. 2002). Highly invasive ants started invading warm regions of Asia recently. The Argentine ant, *Linepithema humile*, was noted in Japan in 1993 (Sugiyama 2000). The red imported fire ant, *Solenopsis invicta*, was noted in Taiwan in 2003, and subsequently in Hong Kong, Macao, and mainland China in 2005 (Zhang et al. 2007). Given the difficulties in eradicating or managing these ants (Silverman and Brightwell 2008), identifying the pathways of dispersal is an important subject to clarify the factors responsible for the recent successive invasions of these ants into Asia and to prevent the spread of them in this region. However, there have been few systematic studies to address the large scale dispersal events of these species (Suarez et al. 2001; Tsutsui et al. 2001; Shoemaker et al. 2006; Corin et al. 2007a), and as for the introduced populations in Asia there have been very few attempts to elucidate their invasion history (Yang et al. 2008).

The Argentine ant is native to South America but has been unintentionally introduced into many parts of the world with Mediterranean-like climates during the last 150 years (Suarez et al. 2001; Wetterer and Wetterer 2006). This species, as well as other highly invasive ants, form supercolonies, within which workers and reproductive castes can move freely among physically separated nests without incurring intraspecific aggression (Holway et al. 2002). Introduced populations segregate into a few large supercolonies that can extend over hundreds to thousands of kilometers (Tsutsui et al. 2000; Giraud et al. 2002), whereas native populations segregate into multiple, mutually incompatible supercolonies that are typically less than hundreds of meters in diameter (Heller 2004; Pedersen et al. 2006). In the introduced range, the Argentine ant almost completely displaces indigenous ants with the numerical advantage gained by the release from intraspecific aggression (Bond and Slingsby 1984; Holway et al. 1998; Holway 1999; Suarez et al. 1999; Touyama et al. 2003; Rowles and O’Dowd 2007).

The social structure of the introduced Argentine ant gives important information on its invasion history: distinct supercolonies may represent independent source populations (Jaquiéry et al. 2005). The Argentine ant, as well as many other ant species, uses its cuticular hydrocarbon profile (the presence or absence of compounds and the relative abundance of shared compounds) as its nestmate recognition cue (Liang and Silverman 2000; Greene and Gordon 2007). Although an environmental factor, namely diet, can modify the cuticular hydrocarbon profile and nestmate recognition under extreme conditions (Liang and Silverman 2000; Silverman and Liang 2001), many studies have suggested that the nestmate recognition cue(s) is heritable (Tsutsui et al. 2000; Giraud et al. 2002; Suarez et al. 2002). Furthermore, gene flow among behaviorally distinct supercolonies is extremely limited (Jaquiéry et al. 2005; Thomas et al. 2006; Pedersen et al. 2006).

The spread of the Argentine ant involves two discrete processes: local colony budding and jump dispersal mediated by humans (Suarez et al. 2001). The latter is the primary mode of spread of the introduced populations. We can retrospectively infer that the huge supercolonies currently formed in areas where introduction occurred decades ago (e.g. Europe and North America: Tsutsui et al. 2000; Giraud et al. 2002) must have been created via numerous jump dispersals of particular populations. Evidence supporting this hypothesis has come from studies in New Zealand, where Argentine ants were introduced recently: introduction(s) from a single source population and its dispersal via human activity may have led to the patchy distribution of a single supercolony in New Zealand (Corin et al. 2007a, b).

Japan is the first, and so far the only country in Asia, where establishment of the Argentine ant has been confirmed (Sugiyama 2000, but see Radchenko 2005 and Corin et al. 2007a). Since its first occurrence in Hatsukaichi City, Hiroshima Prefecture, this species has expanded its distribution primarily through human-mediated jump dispersal, and currently occupies a discontinuous distribution along coastal areas of western Japan (Okaue et al. 2007). The main goal of this study was to gain insight into the invasion history of the Argentine ant populations of Japan. To this end, we studied the behavioral, chemical (in terms of cuticular hydrocarbons), and genetic relationships among these populations. This study also provides an opportunity to better understand the process of formation of huge supercolonies in the introduced range.

Methods

Collection of the Argentine ant

In this study, Argentine ants were collected from the majority of the known colony locations, as of 2005, in Japan.

For behavioral assays, we collected Argentine ants from a total of 16 sites in October 2005 (Figure 1; Table 1). In Port-Island, which is located in Kobe Port, Hyogo Pref. (AREA 2 in Figure 1), intensive collection was conducted since two mutually incompatible supercolonies, which we named the ‘Kobe A’ and ‘Kobe B’ supercolonies, had been detected in preliminary behavioral assays. Kobe Port is one of the top five international ports of Japan. For each collection site, we collected a fragment of an Argentine ant nest (several queens, hundreds of workers, and tens of brood), and maintained it in the laboratory until use. The ants were fed sugar water *ad libitum* and boiled egg every three days.

For chemical analyses, Argentine ants were collected in April or May 2006 from the same sites as used for the behavioral assays. In addition, in November 2007 we collected samples from six sites (MW1-6) at Maya Wharf, which is located in Kobe Port approximately 3 km away from Port-Island (Table 1; Figure 1). At this wharf, two supercolonies, the ‘Kobe C’ supercolony and the ‘Japanese main’ supercolony, were detected in our study in autumn 2006 (already published as Sunamura et al. 2007). Three of the collection sites (MW1-3) were located within the boundary of the Kobe C supercolony and the other three (MW4-6) were located within the boundary of the Japanese main supercolony. For each site, we collected tens of workers and immediately preserved them on dry ice. These ants were maintained at –20˚C until analysis.

For genetic analyses, Argentine ants were collected from a total of 27 sites during 2005-2007 (Table 1). These sites were not exactly the same sites as used for the behavioral or chemical analyses, except for MW1, 3, 4, 5, and TA. Five of the seven collection sites (PI8-12) at Port-Island were located within the boundary of the Kobe A supercolony and the other two (PI13-14) were located within the boundary of the Kobe B supercolony (Sunamura et al. 2007). For each site, tens of workers were collected and preserved in acetone until used for analysis.

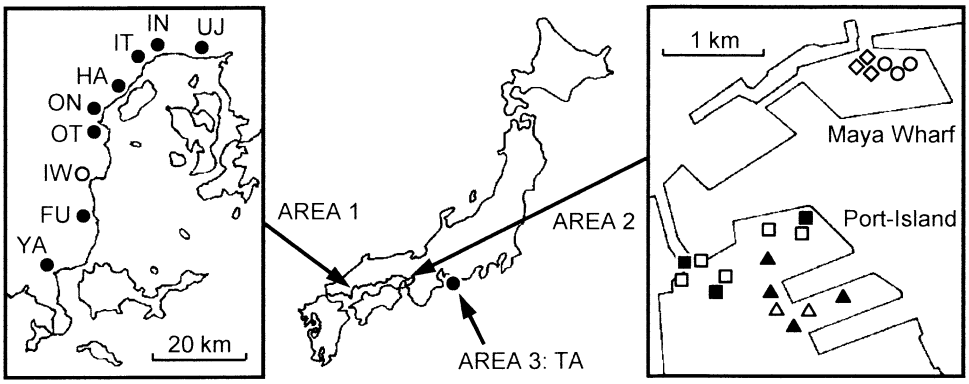

Fig. 1 Map of the collection sites of Argentine ants in Japan. Argentine ants were collected from three areas: AREA 1 (Yamaguchi and Hiroshima Prefectures), AREA 2 (Kobe Port, Hyogo Pref.), and AREA 3 (Tahara, Aichi Pref.). Full names of the localities are shown in Table 1. The behavioral assays in this study and Sunamura et al. (2007) detected four mutually incompatible supercolonies: the Japanese main (circle), Kobe A (square), Kobe B (triangle), and Kobe C (diamond) supercolonies. The sites used for the behavioral assays in this study are shown as filled symbols (site names in AREA 2 are omitted), and sites used only for chemical or genetic analyses are shown as blank symbols (site names are omitted except IW). For the latter sites, the supercolony identities were inferred from our preliminary behavioral assays or Sunamura et al. (2007).

Table 1 Sampling locations for Argentine ants in Japan. Letters in parentheses denote abbreviations of the locality names. Collection sites used for the behavioral assays, chemical analyses, and genetic analyses are shown.

Locality	Site name		
	Behavioral assays	Chemical analyses	Genetic analyses
Yanai, Yamaguchi Pref. (YA)	YA1	YA1	YA2-4
Fuju, Yamaguchi Pref. (FU)	FU1	FU1	FU2, 3
Iwakuni, Yamaguchi Pref. (IW)	-	-	IW1-4
Otake, Hiroshima Pref. (OT)	OT1	OT1	-
Ono, Hiroshima Pref. (ON)	ON1	ON1	-
Hatsukaichi, Hiroshima Pref. (HA)	HA1	HA1	HA2-7
Itsukaichi, Hiroshima Pref. (IT)	IT1	IT1	-
Inokuchi, Hiroshima Pref. (IN)	IN1	IN1	-
Ujina, Hiroshima Pref. (UJ)	UJ1	UJ1	-
Port-Island, Hyogo Pref. (PI)	PI1-7	PI1-7	PI8-14
Maya Wharf, Hyogo Pref. (MW)	-	MW1-6	MW1, 3, 4, 5
Tahara, Aichi Pref. (TA)	TA1	TA1	TA1

Behavioral assays

Live one-on-one aggression tests adapted from earlier studies (Tsutsui et al. 2000; Giraud et al. 2002) were performed. One worker from each of the two nests was introduced into a 5.2 cm diameter plastic dish with fluon-coated sides. The workers were observed until they contacted five times or until five minutes passed after the introduction. When the workers came into contact, their interaction was scored as follows: 1 = ignore or touch (antennation); 2 = avoid; 3 = aggression (lunging, biting, or pulling); 4 = fighting (prolonged aggression). Level 3 and 4 were regarded as aggressive behaviors. Ten replicate trials were performed for each nest pair, and the highest level of aggression for each trial was recorded. The mean highest scores of the ten replicates were used as the aggression indices of the nest pairs. An individual was never used twice.

The aggression tests were conducted in October 2005, within three weeks after the field collection. First, we performed aggression tests between all pairs of the seven nests collected from Port-Island. Since two mutually antagonistic supercolonies (Kobe A and Kobe B supercolonies) were detected by these assays, we then conducted aggression tests between all pairs of the nine nests collected from AREAs 1 and 3 in Figure 1 and two nests from Port-Island (PI2 and PI4 representing the Kobe A and Kobe B supercolonies, respectively) (Table 1, Figure 1). As a control, we also conducted aggression tests between pairs of workers belonging to the same nest for each supercolony.

Chemical analyses

We washed five workers from the same nest in 0.5 ml hexane for 10 min. The solution was loaded onto a Pasteur pipette plugged with 500 mg of silica gel (Wakogel C-200; Wako, Osaka, Japan). The hydrocarbon fraction was then eluted with 5 ml hexane, dried under a nitrogen stream, redissolved in 5 µl hexane, and 2 µl of the solution was analyzed. Gas chromatography was carried out using a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a DB-1 column (30 m × 0.25 mm × 0.25 µm film thickness, J&W Scientific, CA, USA) and interfaced with a CLASS-GC10 Workstation (Shimadzu). Splitless injection was made, and the oven temperature was programmed at 100˚C for 2 min, then increased to 270˚C at 20˚C /min, and then to 310˚C at 5˚C /min, and then held at 310˚C for 21.5 min. The injector and detector were maintained at 270˚C and 320˚C, respectively.

The hydrocarbons were further identified with a GCMS-QP5050A mass spectrometer (Shimadzu) interfaced with a GC-17A gas chromatograph (Shimadzu) equipped with a DB-1 column (30 m × 0.25 mm × 0.25 µm film thickness, J&W Scientific). Mass spectra were obtained using electron impact (EI) mode. The oven temperature was programmed from 60˚C (isothermal for 2 min) to 310˚C at 1˚C /min, and then maintained isothermally for 20 min, Helium was used as the carrier gas, and hydrocarbons were identified based on fragment patterns.

For each of the 22 nests sampled, we conducted the extraction and GC analyses three times, and used the mean value of the relative contribution (i.e. peak area) of the most abundant 25 peaks as a quantitative measure of the cuticular hydrocarbon profile of the nest. We calculated the mean peak areas across all nests analyzed, and determined the peaks that exceeded 1 % as the most abundant. A principal component analysis was performed to evaluate the resemblance of cuticular hydrocarbon profiles among the 22 nests. We also conducted Kruskal-Wallis rank sum tests to test if relative contribution of each peak differed among supercolonies. The statistical analyses were performed with R (R Development Core Team 2007).

Genetic analyses

DNA was extracted from 15 workers from each nest using a phenol-chloroform extraction method (Sambrook et al. 1989) and dissolved in 50 µl Low-TE buffer. Two microsatellite loci (Lhum-11 and Lhum-19: Krieger and Keller 1999) from the nuclear genome were amplified by polymerase chain reaction (PCR). Each 10 µl reaction mixture consisted of 0.5 µl template DNA, 2 mM Tris-HCl, 10 mM KCl, 0.1 mM EDTA, 0.1 mM DTT, 0.05% Tween 20, 0.05% Nonidet P-40, 5% Glycerol, 1.5 mM $MgCl_2$, 200 mM each of dNTPs, 0.25 µM each of reverse and forward primers, and 0.2 unit of *TAKARA Taq* DNA polymerase (TAKARA, Otsu, Japan). PCR was carried out in a PC-800 temperature control system (ASTEC, Fukuoka, Japan) following the conditions specified by Krieger and Keller (1999). The PCR product was electrophoresed through an 8% polyacrylamide gel at 300 V for 1.5-2 h, and the DNA bands were visualized by silver staining.

To assess the genetic differences between the nests, F_{st} (Wright 1951) was calculated between each nest pair using GENEPOP ver.3.1 (Raymond and Rousset 1995), and a hierarchical cluster analysis was carried out using STATISTICA (StatSoft Inc., Tulsa, OK, USA), using F_{st} as a distance index and UPGMA (Unweighted Pair Group Method with Arithmetic mean: Sneath and Sokal, 1973). We also calculated Wright's *F*-statistics (Wright 1951) using GENETIC DATA ANALYSIS (Lewis and Zaykin 1998) ver.1.1 regarding the supercolonies as subpopulations. The 95% confidence intervals were estimated with 1000 bootstrap resamplings.

Results

Behavioral assays

Aggression tests revealed the existence of three mutually incompatible supercolonies. The seven nests collected from Port-Island were segregated into the Kobe A and Kobe B supercolonies. The colonies collected at PI1-3 were assigned to the Kobe A supercolony, and the colonies collected at PI4-7 were assigned to the Kobe B supercolony (Figure 1). The eight nests collected from AREAs 1 and 3 in Figure 1 were assigned to the third supercolony, the 'Japanese main' supercolony. The population from Iwakuni (Table 1, Figure 1) was not used in the behavioral assays of this study, but was found to belong to the Japanese main supercolony by preliminary assays. Aggressive behavior was commonly observed between pairs of workers from different supercolonies, whereas avoidance or aggressive behavior was absent between pairs of workers from the same supercolony or nest (Figure 2).

Chemical analyses

Field-collected Argentine ant workers contained a complex mixture of linear, methyl-branched, dimethyl-branched, and trimethyl-branched alkanes, ranging from C17 to C37, as well as an alkene (Table 2). Hydrocarbons with 33, 35, and 37 carbons in chain length had very similar branches. Significant differentiation among supercolonies was detected for relative proportions of many hydrocarbons (14 out of 25 hydrocarbon peaks; $P < 0.05$, Kruskal-Wallis rank sum test).

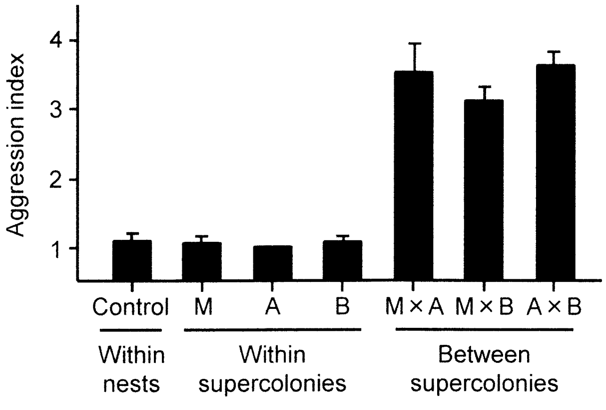

Fig. 2 Results of the aggression tests within and among Argentine ant nests collected in Japan. Interactions of workers were scored 1 (ignore or touch), 2 (avoid), 3 (aggression), or 4 (fight). Mean + SD aggression indices are shown. M, A, and B denote the Japanese main supercolony, Kobe A supercolony, and Kobe B supercolony, respectively.

In the principal component analysis, the first, second, and third principal components represented 80.4 % of the total variance (48.4 %, 19.1 %, and 12.9 %, respectively). On the plots of scores for either two of the first to third principal components, the four behaviorally distinct supercolonies were separated (Figure 3). The cuticular hydrocarbon profiles of the Japanese main supercolony appeared to be somewhat divergent among disjunct distributions, with the profiles of the workers collected from nearly located sites in Maya Wharf (MW4-6) clustering together. Many hydrocarbons had large contributions to the main scores: the correlation coefficient between a hydrocarbon and either of the first to third principal components was > 0.6 or < -0.6 for 22 out of 25 hydrocarbons (the exception was peak number 1, 2 and 21 in Table 2). For example, the hydrocarbons which had highly positive correlations (0.71 to 0.95) to the first principal component were peak number 9, 13, 19, 20, 24, and 25 in Table 2, namely trimethylalkanes longer than 31 carbons in chain length. On the other hand, the hydrocarbons which had highly negative correlations (-0.68 to -0.96) to the first principal component were peak number 6, 7, 10, 11, 12, 15, 16, 17, and 18 in Table 2, mainly monoemthyl- and dimethylalkanes ranging in chain length from 31 to 35 carbons. Correlation coefficients of other hydrocarbons to the first principal component were -0.46 to 0.16.

Genetic analyses

A total of nine and eight alleles were found from Lhum-11 and Lhum-19 loci, respectively (Table 3). Significant deviations of the genotype frequencies from the Hardy-Weinberg equilibrium ($P < 0.05$) were not detected except three nests (HA5 in Lhum-11; MW1 and MW3 in Lhum-19). The estimated value (95 % confidence interval) of *F* statistics regarding the four supercolonies as subpopulations were: F_{is}, 0.120 (0.085-0.150); F_{it}, 0.404 (0.388-0.420); F_{st}, 0.322 (0.280-0.367). Each statistic exhibited significant ($P < 0.05$) deviations from 0. The behaviorally distinct supercolonies were genetically

Table 2 Cuticular hydrocarbon profiles of the Argentine ant populations of Japan. Percentages of the abundant 25 peaks (Mean ± SD) are shown for each supercolony.

Peak	Compound	Japanese main	Kobe A	Kobe B	Kobe C	P^a
1	n-C17	2.60 ± 1.56	1.70 ± 1.14	2.08 ± 1.33	2.57 ± 1.12	0.71
2	C17-1	5.14 ± 2.07	4.25 ± 1.65	4.35 ± 2.22	6.53 ± 1.91	0.67
3	n-C27	3.18 ± 0.99	5.83 ± 1.45	5.08 ± 1.48	0.75 ± 0.44	0.051
4	n-C28	0.97 ± 0.38	1.50 ± 0.32	1.35 ± 0.57	1.03 ± 0.46	0.19
5	n-C29	2.03 ± 0.67	3.13 ± 0.64	3.32 ± 0.98	2.27 ± 0.69	0.076
6	n-C31	0.96 ± 0.43	1.56 ± 0.30	1.26 ± 0.28	1.57 ± 0.16	0.19
7	13- + 15-MeC31	0.89 ± 0.32	1.33 ± 0.34	1.60 ± 0.18	1.93 ± 0.24	< 0.005
8	3- + 5-MeC31	1.32 ± 0.46	1.30 ± 0.48	2.18 ± 0.61	1.56 ± 0.22	< 0.01
9	5,13,15- + 5,13,17-triMeC31	1.95 ± 0.45	1.52 ± 0.35	2.24 ± 0.32	0.82 ± 0.15	0.085
10	13- + 15- + 17-MeC33	1.80 ± 0.57	2.70 ± 0.65	3.00 ± 0.54	4.53 ± 0.50	< 0.005
11	11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-diMeC33	1.39 ± 0.33	2.64 ± 0.26	1.73 ± 0.28	5.77 ± 0.55	< 0.05
12	5,15- + 5,17- diMeC33	2.77 ± 0.34	2.19 ± 0.17	3.72 ± 0.54	9.90 ± 1.52	< 0.005
13	5,13,17- + 5,15,17- + 5,13,19- + 5,15,19-triMeC33	5.76 ± 0.97	3.01 ± 0.35	5.12 ± 0.98	1.00 ± 0.12	0.055
14	3,13,15- + 3,13,17- + 3,13,19- + 3,15,17- + 3,15,19-triMeC33	1.68 ± 0.31	3.32 ± 0.68	2.18 ± 0.17	1.78 ± 0.32	0.18
15	7,11,15- + 7,11,17- + 7,13,15- + 7,13,17-triMeC33	0.95 ± 0.57	2.00 ± 0.21	1.12 ± 0.50	2.16 ± 0.26	0.15
16	13- + 15- + 17-MeC35	2.46 ± 0.82	2.74 ± 0.39	3.16 ± 0.73	3.91 ± 0.41	< 0.005
17	11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-diMeC35	2.76 ± 0.44	4.76 ± 0.92	2.84 ± 0.33	8.00 ± 0.83	< 0.05
18	5,15- + 5,17-diMeC35	4.98 ± 0.62	4.22 ± 0.57	4.27 ± 0.71	10.05 ± 1.40	< 0.005
19	5,13,17- + 5,13,19- + 5,15,17- + 5,15,19-triMeC35	15.51 ± 2.98	3.07 ± 0.65	8.29 ± 1.79	0.83 ± 0.18	< 0.05
20	3,13,15- + 3,13,17- + 3,13,19- + 3,15,17- + 3,15,19-triMeC35	3.29 ± 0.56	3.05 ± 0.65	2.40 ± 0.37	1.18 ± 0.27	< 0.001
21	13- + 15- + 17- + 19-MeC37	1.94 ± 0.68	1.41 ± 0.16	2.46 ± 0.74	1.42 ± 0.13	< 0.05
22	11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-diMeC37	3.54 ± 0.44	5.17 ± 0.91	3.09 ± 0.57	3.76 ± 0.40	0.062
23	5,15- + 5,17-diMeC37	2.64 ± 0.58	4.13 ± 0.63	1.65 ± 0.44	1.68 ± 0.25	< 0.005
24	5,13,17- + 5,13,19- + 5,15,17- + 5,15,19-triMeC37	7.87 ± 1.61	0.88 ± 0.53	3.19 ± 0.81	0.07 ± 0.14	< 0.05
25	3,13,15- + 3,13,17- + 3,13,19- + 3,15,17- + 3,15,19-triMeC37	1.76 ± 0.64	0.76 ± 0.45	0.79 ± 0.46	0	< 0.05

[a] Kruskal-Wallis rank sum test among four supercolonies.

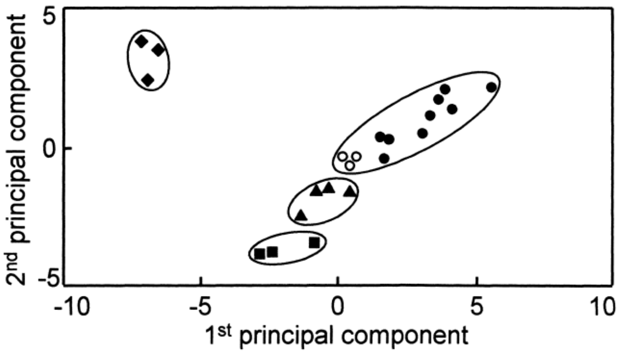

Fig. 3 Principal component maps of cuticular hydrocarbons from the Japanese populations of the Argentine ant. Similarity of the cuticular hydrocarbon profiles was evaluated among 22 nests, using the relative abundance of 25 hydrocarbon peaks listed in Table 2. Circles, squares, triangles, and diamonds denote nests from the Japanese main, Kobe A, Kobe B, and Kobe C supercolony, respectively. Open circles denote Maya Wharf population of the Japanese main supercolony (i.e. nests MW4-6 in Table 1).

different not only in the frequencies of shared alleles but also in the presence (e.g. allele 3 of Lhum-11 for the Kobe B supercolony, and allele 1 of Lhum-19 for the Kobe A supercolony: Table 3) or absence (e.g. allele 6 and 7 of Lhum-11, and allele 4 and 7 of Lhum-19 for the Kobe C supercolony) of specific alleles.

The cluster analysis using F_{st} between the collection sites divided the Argentine ant nests into five clusters (I-V) at a distance level of 0.1 (Figure 4). These clusters generally coincided with the four behaviorally distinct supercolonies. The only exception was that the nests of the Japanese main supercolony in Maya Warf (MW4 and MW5) were not included in the cluster (II) of the Japanese main supercolony, but formed a cluster (III) with the nests of the Kobe B supercolony. Alleles found from the Maya Wharf nests of the Japanese main supercolony were a subset of the alleles found from the remaining nests of this supercolony, but the allele frequencies were different between the two groups (Table 3). The F_{st} between nests from the same supercolony was 0.080 ± 0.099 (-0.045 to 0.43) when the values between the Maya Wharf nests and the other nests of the Japanese main supercolony (0.24 ± 0.070) were included, and 0.040 ± 0.058 (-0.045 to 0.25) when these values were excluded. The F_{st} between nests from distinct supercolonies was 0.36 ± 0.13 (0.061 to 0.60).

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Table 3. Allele frequencies of two microsatellite loci for the four Japanese supercolonies of the Argentine ant. As for the Japanese main supercolony, data for the Maya Wharf nests (MW4 and MW5 in Table 1) are shown in parentheses.

	allele	Japanese main n = 270 (30)	Kobe A n = 75	Kobe B n = 30	Kobe C n = 30
Lhum-11	1	-	0.020	-	-
	2	-	0.053	0.20	-
	3	-	-	0.17	-
	4	0.0019 (-)	-	-	-
	5	-	-	0.033	-
	6	0.27 (0.17)	0.85	0.20	-
	7	0.011 (-)	0.080	0.13	-
	8	0.16 (0.65)	-	0.18	-
	9	0.56 (0.18)	-	0.083	1.0
Lhum-19	1	-	0.42	-	-
	2	-	0.020	-	-
	3	0.0037 (0.017)	0.35	0.067	0.18
	4	0.16 (0.17)	0.027	0.22	-
	5	0.11 (0.033)	-	0.017	0.56
	6	0.61 (0.35)	-	0.40	0.083
	7	0.0019 (-)	0.0067	0.050	-
	8	0.22 (0.43)	0.17	0.25	0.18

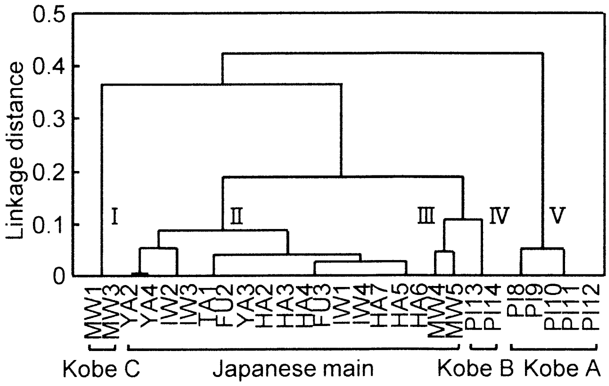

Fig. 4 Genetic relationship among the Argentine ant nests collected from 27 locations in Japan (listed in Table 1). A hierarchical cluster analysis was performed based on the F_{st} between the nests calculated from microsatellite data. Nest names are the same as listed in Table 1.

Discussion

Social structure of the introduced Argentine ant populations in Japan

So far, four behaviorally distinct supercolonies of the Argentine ant have been detected from Japan. The behavioral assays in the current study detected the Japanese main, Kobe A, and Kobe B supercolonies. Because of the finding of two supercolonies from Kobe Port, we investigated detailed distribution of the supercolonies there, and discovered the fourth supercolony, Kobe C, in Maya Wharf (Sunamura et al. 2007). The present study is one of the very few studies that investigated the behavioral relationships among Argentine ant populations in countries where introduction occurred recently. The pattern of the supercolony distribution in Japan (i.e. existence of one large supercolony and several smaller supercolonies) is similar to the other parts of the world where introduction took place decades ago (Tsutsui et al. 2000; Giraud et al. 2002). However, our results is surprising, because only one supercolony has been found from New Zealand, where Argentine ants were first discovered about the same time as Japan (Corin et al. 2007b).

The plausibility of the results of our behavioral assays was confirmed by the results of the chemical analyses: relative abundance of the cuticular hydrocarbons was similar within the same, but divergent among distinct supercolonies (Figure 3). So far, cuticular hydrocarbons of Argentine ants have been investigated only for a few supercolonies in North America and the dominant supercolony in Europe (e.g. Liang et al. 2001; de Biseau et al. 2004; Buczkowski et al. 2005; Torres et al. 2007). The present study reinforces the knowledge with data for as many as four supercolonies. Argentine ant supercolonies in Japan had almost the same hydrocarbons as the aforementioned foreign supercolonies (Liang et al. 2001; de Biseau et al. 2004). Relative abundance of many of the hydrocarbons was significantly different among supercolonies, and contributed greatly to the principal components in the principal component analysis. These results suggest that the differences of cuticular hydrocarbon profiles among supercolonies are attributed to the relative abundance of many hydrocarbons, not a few specific hydrocarbons. However, the smallest hydrocarbons analyzed in this study (i.e. n-C17 and C17-1: Table 3) had not contributed greatly to the difference of cuticular hydrocarbon profiles among the Argentine ant populations of Japan. These hydrocarbons may not play important roles in discriminating members and non-members of supercolonies.

Nests belonging to the same behaviorally defined supercolony were genetically more similar to each other than nests belonging to distinct supercolonies (Figure 4), and the high F_{st} value inferred from the genotype data suggests considerable genetic differentiation among the four supercolonies. These results are consistent with the previous studies that suggested that the nestmate recognition in the Argentine ant is genetically based (Tsutsui et al. 2000; Giraud et al. 2002). The exception to this pattern found in the nests from Maya Wharf (Figure 4) might be due to the founder effect. The bottlenecks and the genetic drift at the occasions of introduction or jump dispersal can lead to relatively high divergence rates in some microsatellite loci between the source and the introduced population. Further genetic research using more loci and more conserved markers such as mitochondrial DNA is necessary.

The genetic analysis showed that the Kobe A supercolony was the most genetically distinct supercolony among the four Japanese supercolonies (Figure 4), but the chemical analysis showed that the Kobe C supercolony had the most distinct cuticular hydrocarbon profiles (Figure 3). Therefore, supercolonies that were more closely related did not necessarily have more similar cuticular hydrocarbon profiles. In several of the previous studies, clear relationship was not detected between the levels of intraspecific aggression (which correlate with the resemblance of cuticular hydrocarbon profiles: Suarez et al. 2002) and genetic relatedness (Tsutsui et al. 2000; Buczkowski et al. 2004). These results were possibly due to the use of neutral microsatellites as genetic markers, which may not be involved in the process of nestmate recognition. This might also be the case for the Japanese supercolonies.

Invasion history of the introduced Argentine ant of Japan

The behavioral, chemical, and genetic differentiation among the introduced Argentine ants of Japan suggests that each of the four Japanese supercolonies represents independent source populations. Since simultaneous introduction of more than one supercolony is unlikely, Japan may have received at least four independent introductions recently. The number of alleles detected from Japan (nine alleles for Lhum-11 and eight alleles for Lhum-19, all supercolonies summed) matches those detected from California (11 alleles for Lhum-11: Tsutsui et al. 2000), southeastern U.S. (eight alleles for Lhum-11 and seven alleles for Lhum-19: Buczkowski et al. 2004) and Europe (eight alleles for Lhum-11: Giraud et al. 2002), where multiple introductions have been suggested, but considerably higher than detected from New Zealand (four alleles for Lhum-11: Corin et al. 2007b).

The current study appears to represent the recent trend of increasing invasions of invasive ant species in Asia. The red imported fire ant has invaded several regions of Asia (Zhang et al. 2007). Furthermore, Yang et al. (2008) suggested that the introduced populations of this species in Taiwan derived from two introductions. Establishment of the Argentine ant in Asia has not been reported other than from Japan, but Thailand and Singapore were recorded as the source for Argentine ants transported to New Zealand (Corin et al. 2007a). Radchenko (2005) also recorded a male individual of the Argentine ant from North Korea. Our results, combined with these reports, yield the urgent need to investigate the exact distribution of invasive ants in Asia, and identify the factor(s) responsible for the recent multiple invasions.

Kobe port may have received introduction of Argentine ants at least three times from abroad, since the Kobe A-C supercolonies have not been detected out of Kobe Port (Figure 1). To our knowledge, presence of more than two supercolonies within such a small area like Kobe Port has never been reported from any other introduced areas of the world. However, multiple invasions of different supercolonies can happen to areas with active human commerce such as harbors and airports. To date, social structure of the Argentine ant has been studied mainly within areas where introduction took place decades ago (e.g. Tsutsui et al. 2000; Giraud et al. 2002). In such areas, Argentine ants are now distributed over hundreds to thousands of kilometers, and aggression tests have been conducted at much larger spatial scales than conducted in the present study. With detailed samplings, patterns of invasion similar to the case of Kobe Port might be detected from other parts of the world.

In contrast to the Kobe A-C supercolonies, the Japanese main supercolony was found from almost all of the study locations (Figure 1). This supercolony must have expanded its distribution through human-mediated jump dispersal. The jump dispersal events can include both repeated introductions from abroad and dispersals from older domestic distributions to new locations. Further attempt should be made to distinguish these two patterns. However, since many of the distributions of the Japanese main supercolony are far from areas where Argentine ants can be introduced directly from abroad (e.g. ports and airports), these distributions should have been created mainly via jump dispersals from older domestic distributions.

Formation of huge supercolonies in the introduced range

Our results on the Japanese main supercolony show that an Argentine ant supercolony can expand its size via human-mediated jump dispersal. This is consistent with the previous behavioral and genetic studies on the Argentine ants of New Zealand (Corin et al. 2007a, b). Previous laboratory experiments showed that environment can influence cuticular hydrocarbons and intraspecific aggression (Liang and Silverman 2000; Silverman and Liang 2001). However, the resemblance of cuticular hydrocarbon profiles among disjunct populations of the Japanese main supercolony suggests that the jump dispersal events (i.e. changes in environment) did not cause extreme changes in the cuticular hydrocarbon profiles. Furthermore, internationally disjoint populations (i.e. North American, European, and Japanese populations), which may inhabit considerably different environments, had almost identical hydrocarbons, suggesting that these populations had not obtained significant amount of hydrocarbons unique to each habitat. However, the cuticular hydrocarbon profiles of the Japanese main supercolony appeared to be somewhat divergent among disjunct localities (Figure 3). Further detailed study is necessary to evaluate the divergence of cuticular hydrocarbon profiles within a nest, among nests, and among disjunct distributions of one supercolony. The bottlenecks and/or changes in environment concomitant to the jump dispersal events might have affected the genetic and/or environmental factors that determine the cuticular hydrocarbon profiles, to some extent.

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