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RESEARCH ARTICLE

Intercontinental union of Argentine ants: behavioral relationships among introduced populations in Europe, North America, and Asia

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Abstract Many invasive ants, including the Argentine ant *Linepithema humile*, form expansive supercolonies, within which intraspecific aggression is absent. The behavioral relationships among introduced Argentine ant populations at within-country or within-continent scales have been studied previously, but the behavioral relationships among intercontinental populations have not been examined. The present study investigated the levels of aggression among intercontinental Argentine ant populations by transporting live ants from Europe and California to Japan and conducting aggression tests against Japanese populations. Workers from the dominant supercolonies of Europe and California did not show aggressive behavior toward workers from the dominant supercolony of Japan, whereas they fought vigorously against workers from minor supercolonies. The three massive supercolonies, together with Argentine ants from Macaronesia, may be the largest non-aggressive unit formed by a social insect species in which intraspecific aggression exists. Absence or low levels of aggression at transcontinental scale, which may have derived from low genetic variation, may help introduced Argentine ants maintain expansive supercolonies. The lack of aggression implies possible frequent exchanges of individuals among the intercontinental populations mediated by human activities.

Keywords Biological invasions · Intraspecific aggression · Invasion history · *Linepithema humile* · Supercolony

Introduction

The success of invasive ants can be partly attributed to the absence of intraspecific aggression at large spatial scales, or the unusual social structure called supercolonies, within which individuals can move freely among physically separated nests (Holway et al., 2002). Native to South America, the Argentine ant, *Linepithema humile*, has been unintentionally introduced to all continents except Antarctica (Suarez et al., 2001). Introduced Argentine ant populations are renowned for forming much larger and fewer supercolonies than native populations (Tsutsui et al., 2000; Giraud et al., 2002; Heller, 2004; Pedersen et al., 2006). The most conspicuous examples of colossal Argentine ant supercolonies are the 'European main' supercolony, which extends over 6,000 km along the Mediterranean coast (Giraud et al., 2002), and the 'Californian large' supercolony, which extends over 900 km along coastal California (Tsutsui et al., 2000).

Behavioral relationships among beyond-ocean introduced populations of the Argentine ant are scarcely known. Recently, Wetterer and Wetterer (2006) found that Argentine ants from the European main supercolony and Macaronesian archipelagos were mutually non-aggressive. In our previous study (Sunamura et al., 2009, in press), we analyzed cuticular hydrocarbons (nestmate recognition cues: Liang and Silverman, 2000) of the introduced populations of Japan, and

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found that the gas chromatogram for the dominant supercolony in Japan (Fig. 1) closely resembled those reported for the dominant supercolonies in California and Europe (Liang et al., 2001; de Biseau et al., 2004). The present study investigated actual behavioral relationships among these intercontinental Argentine ant populations with aggression tests. The results provide insights into the mechanisms underlying the formation of expansive supercolonies in the introduced range, and the invasion history of Argentine ants.

Materials and methods

For this study, fragments of seven Argentine ant supercolonies were collected. In Europe, the European main supercolony and the 'Catalonian' supercolony, which extends over 700 km along the Iberian Mediterranean coast (Giraud et al., 2002), were collected in October 2007. In the USA, a fragment of the Californian large supercolony was collected in July 2007. In Japan, fragments of the 'Japanese main' supercolony, which occurs along 400 km of the coast of western Japan and the 'Kobe A-C' supercolonies, located in Kobe Port only (Sunamura et al., 2007, 2009, in press), were collected in November 2007. For each collection site listed in Table 1, a single laboratory colony was established and maintained until use. The ants were fed artificial diet (Bhatkar and Whitcomb, 1970) twice a week in Europe, scrambled eggs, crickets, a protein solution and sugar water three times a week in the USA, before transported to Japan. In Japan, the ants were fed boiled egg and sugar water every 3 days.

Traditional one-on-one aggression tests were conducted. One worker from each of two colonies was introduced into a 5.2 cm diameter plastic dish. Interactions between the workers were observed for 10 min and scored as follows: 0 = ignore, 1 = antennation, 2 = avoid, 3 = aggression (lunging, pulling or biting), and 4 = fight (prolonged aggression). Scores 3 and 4 were regarded as aggressive behaviors. Six replicates of aggression tests were conducted for each colony pair. The highest score observed during the 10 min was used as the aggression score for each trial.

In November 2007, behavioral assays were conducted that tested the two European supercolonies against the four Japanese supercolonies. In February 2008, behavioral assays were conducted that tested the Californian large supercolony against the four Japanese supercolonies. These assays were conducted within 48 h after the receipt in Japan of Argentine ants from Europe or USA. Aggression tests were also conducted for all the pairs of the colonies collected in Japan, and between pairs of workers belonging to the same colony for all the colonies collected in Japan.

Results

In the behavioral assays that tested the European main and Californian large supercolonies against the Japanese main supercolony (assays 1), workers interacted through antennation or showed no mutual interest, and avoidance or aggressive behaviors were never observed (Table 2). This was the same behavioral pattern as observed in the assays conducted between workers from the same colonies (assays 2) and colonies belonging to the same supercolony (assays 3). In contrast, avoidance or aggressive behaviors were commonly detected in the assays conducted for the rest of supercolony pairs (assays 4). Aggression scores did not differ significantly among assays 1–3 (Steel-Dwass test, $P > 0.8$ for all pairs of assays 1–3), but these scores were significantly lower than the scores for assays 4 (Steel-Dwass test, $P < 0.001$ for all pairs between assays 1–3 and assays 4).

Discussion

The present data suggest that the international disjoint non-aggressive population found by Wetterer and Wetterer (2006) is much larger than recognized previously. The aggregation of the three large intercontinental supercolonies and Macaronesian populations may be the largest non-aggressive unit formed by a social insect species in which intraspecific aggression exists. In fact, the enormous extent of this population is paralleled only by that of the human society. Because both international and within-country dispersals of the Argentine ant heavily depend on human activity (Suarez et al., 2001), it is humans that have created this great non-aggressive ant population.

The most plausible hypothesis that explains the absence of aggression among the three intercontinental supercolonies may be that they are genetically similar and thus have similar or identical heritable recognition cues. Whereas nestmate recognition and aggression may be genetically based (Tsutsui et al., 2000, 2003; Pedersen et al., 2006), they can sometimes be influenced by environment (Liang and Silverman, 2000). Therefore, the consistency of the present behavioral data may merit further examination. However, the level of aggression among the three intercontinental supercolonies, if any, may be low, because environmental factors may not mask high levels of aggression derived from strong genetic differences (Buczkowski et al., 2005). Indeed, Tsutsui et al. (2001) showed that many of the introduced Argentine ant populations across the world are genetically similar. Low levels of both genetic variation and intraspecific aggression at the global scale could help introduced Argentine ants maintain huge supercolonies.

The present results show that Argentine ants from several enormous international populations can accept each

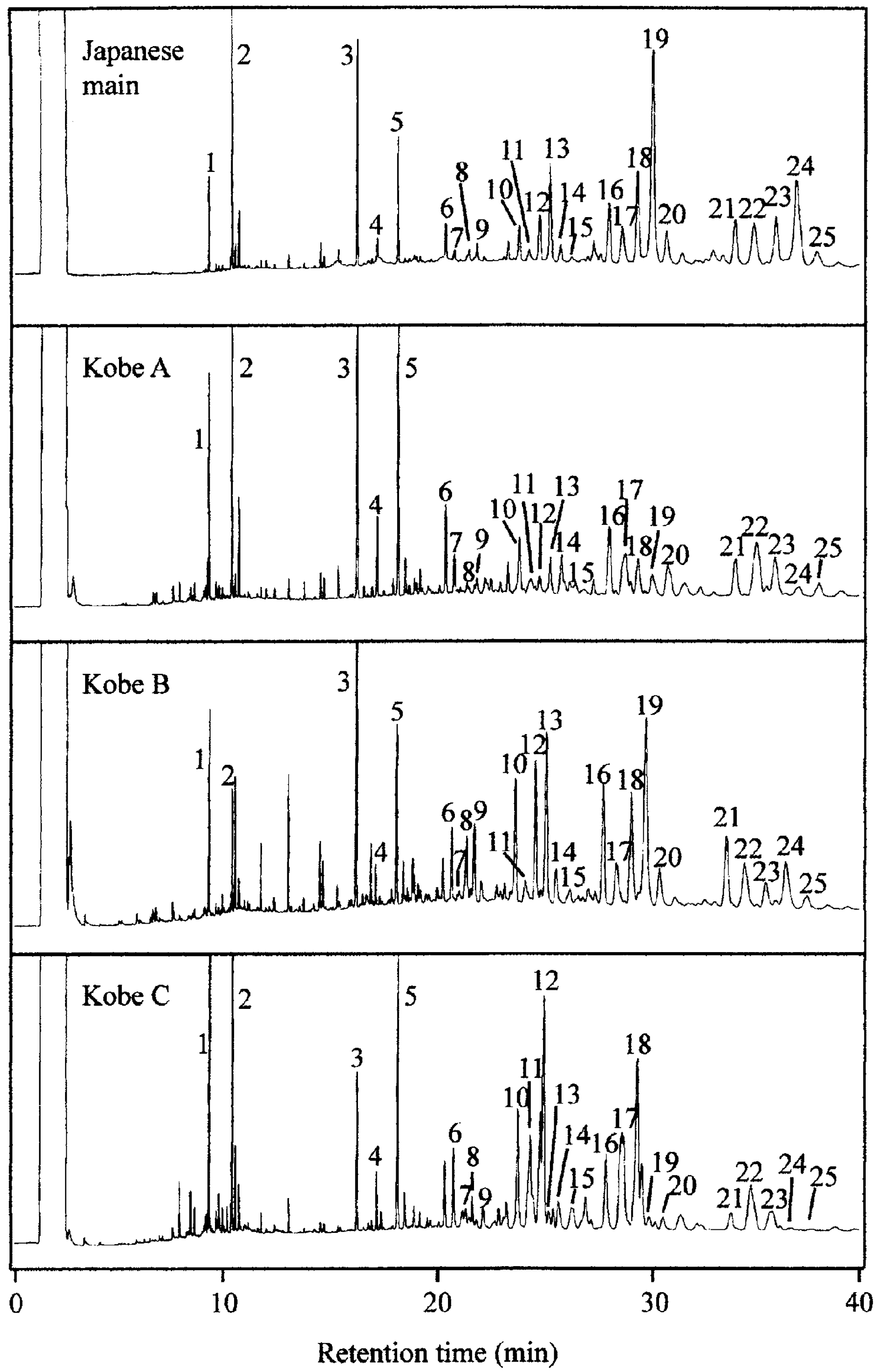

Fig. 1 Gas chromatograms for cuticular hydrocarbons of the four Japanese Argentine ant supercolonies. The chromatogram for the Japanese main supercolony closely resembles those reported for the Californian large and European main supercolonies (Liang et al., 2001; de Biseau et al., 2004). Main peaks are: *1* *n*-C17, *2* C17-1, *3* *n*-C27, *4* *n*-C28, *5* *n*-C29, *6* *n*-C31, *7* 13- + 15-MeC31, *8* 3- + 5-MeC31, *9* 5,13,15- + 5,13,17-triMeC31, *10* 13- + 15- + 17-MeC33, *11* 11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-diMeC33, *12* 5,15- + 5,17-diMeC33, *13* 5,13,17- + 5,15,17 - + 5,13, 19 ± 5,15,19-triMeC33, *14* 3,13,15- + 3,13,17- + 3,13,19- + 3,15,17- + 3,15,19-triMeC33, *15* 7,11,15- + 7,11,17 - + 7,13,15 - + 7,13,17-triMeC33, *16* 13- + 15 - + 17-MeC35, *17* 11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-diMeC35, *18* 5,15- + 5,17-diMeC35, *19* 5,13,17- + 5,13,19- + 5,15,17- + 5,15,19-triMeC35, *20* 3,13,15- + 3,13,17- + 3,13,19- + 3,15, 17- 3,15,19-triMeC35, *21* 13- + 15- + 17- + 19-MeC37, *22* 11,17- + 11,19- + 13,17- + 13,19- + 15,17- + 15,19- + 17,19-di MeC37, *23* 5,15- + 5,17-diMeC37, *24* 5,13,17- + 5,13,19- + 5,15, 17- + 5,15,19-triMeC37, *25* 3,13,15- + 3,13,17- + 3,13,19- + 3,15,17- + 3,15,19-triMeC37. Conditions for gas chromatography are described in Sunamura et al. (2009, in press)

Table 1 Information on the nine Argentine ant colonies studied

Colony	Locality	Position
Japanese main (1)[a]	Kuroiso, Iwakuni, Japan	34°06′15″N and 132°12′01″E
Japanese main (2)[a]	Eastern Maya Wharf, Kobe, Japan	34°41′49″N and 135°13′47″E
Japanese main (3)[a]	Konohana, Osaka, Japan	34°40′32″N and 135°25′12″E
Kobe A	Kita-Kouen, Port-Island, Kobe, Japan	34°40′24″N and 135°12′18″E
Kobe B	Naka-Futou, Port-Island, Kobe, Japan	34°40′19″N and 135°13′16″E
Kobe C	Western Maya Wharf, Kobe, Japan	34°41′37″N and 135°13′25″E
Californian large	Davis, Yolo County, California, USA	38°30′00″N and 121°41′00″W
European main	Cerdanyola, Barcelona, Spain	41°29′30″N and 2°08′48″E
Catalonian	Sant Cugat del Vallès, Barcelona, Spain	41°28′28″N and 2°04′34″E

[a] The Japanese main supercolony was collected from three localities

Table 2 Results of the aggression tests

	Jpn main (1)	Jpn main (2)	Jpn main (3)	Kobe A	Kobe B	Kobe C
Jpn main (1)	0.83 ± 0.41	1.0 ± 0.0	0.83 ± 0.41	3.3 ± 0.82	2.7 ± 0.82	3.5 ± 0.84
Jpn main (2)		0.67 ± 0.52	0.83 ± 0.41	3.8 ± 0.41	3.5 ± 0.84	3.7 ± 0.52
Jpn main (3)			0.67 ± 0.52	3.8 ± 0.41	3.3 ± 0.52	3.8 ± 0.41
Kobe A				0.83 ± 0.41	4.0 ± 0.0	3.5 ± 0.84
Kobe B					1.0 ± 0.0	3.7 ± 0.52
Kobe C						0.83 ± 0.41
Californian large	0.83 ± 0.41	0.67 ± 0.52	0.67 ± 0.52	3.7 ± 0.52	3.7 ± 0.52	4.0 ± 0.0
European main	1.0 ± 0.0	–	–	3.0 ± 0.63	3.0 ± 0.0	3.0 ± 0.89
Catalonian	3.2 ± 1.6	–	–	3.7 ± 0.82	3.7 ± 0.82	2.8 ± 1.6

Interactions of workers were scored; 0 (ignore), 1 (antennation), 2 (avoid), 3 (aggression), or 4 (fight). Mean ± SD aggression scores of six replicates for each pair of colonies are shown

Jpn Japanese

other without causing intraspecific aggression. There might have been exchanges of individuals, including reproductive castes, among these populations in the past mediated by human activities. Whereas an Argentine ant supercolony in a given area can be derived from introduction and dispersal of a particular source population, it can also be derived from multiple introductions of mutually non-aggressive populations (Björkman-Chiswell et al., 2008). The present results show that the latter pattern can be the case for a considerable portion of the world.

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